# WELCOME TO OZ BIOSCIENCES' CATALOG

Progress in understanding biological systems and the development of new medicines are among the greatest endeavours of our era.

OZ Biosciences is dedicated to creating innovative technologies that accelerate biological discovery. We provide rising generations of research reagents based on molecular delivery systems to serve and assist the life science community in its mission.

We are always looking for new ways to bring Transfection & Transduction Solutions and our R&D teams have significantly expanded our comprehensive line of transfection and transduction tools. These new reagents and innovative technologies are introduced in this new catalog with our cutting-edge products.

Our reagents are based on 5 proprietary technologies:

- The Magnetofection<sup>TM</sup> technology is based on magnetic nanoparticles to transfect or to transduce cells. It is the perfect solution for hard-to-transfect and primary cells as well as for *in vivo* gene delivery. Tailored reagents are available including the popular NeuroMag, specific for neurons transfection & the whole range of ViroMag reagents, to enhance infection.
- The CHAMP<sup>TM</sup> technology, with the design and synthesis of a novel patented polymer which is biocompatible, cleavable, pH responsive and bi-functional.
   This polymer-based transfection technology is an optimized delivery system that allows high efficiency with low cellular stress thanks to improved delivery mechanisms.

- The Lipofection technology is based on our patented biodegradable cationic lipids that Trigger Endosomal Escape. This classical method is ideal to transfect cell lines.
- The **3D** transfection technology is an innovative technology specifically developed to directly transfect cells cultured in 3D matrices (Hydrogel / Scaffold).
- The i-MICST<sup>TM</sup> technology (integrated Magnetic Immuno-Cell Sorting and Transfection/Transduction) combines cell isolation and genetic modification in one simple, efficient and reliable system. The Viro-MICST<sup>TM</sup> reagent allows the efficient and specific infection of target cells directly on magnetic cell-purification columns.

Enjoy exploring our product portfolio in this catalog and visit our website to learn about all the tools we provide.

Our team is wishing you a flourishing and passionate research!

OZ BIOSCIENCES

THE ART OF DELIVERY SYSTEMS

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#### TECHNICAL INFORMATIONS

Protocols, results, MSDS and product information sheets are available at: www.ozbiosciences.com

If you have any questions, our highly trained Technical Representatives will be more than happy to assist you: <a href="tech@ozbiosciences.com">tech@ozbiosciences.com</a>

To find the right transfection reagent for your application, please check out our website and consult our **Reagent Finder** and **Citation database**.



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#### Transfection tools

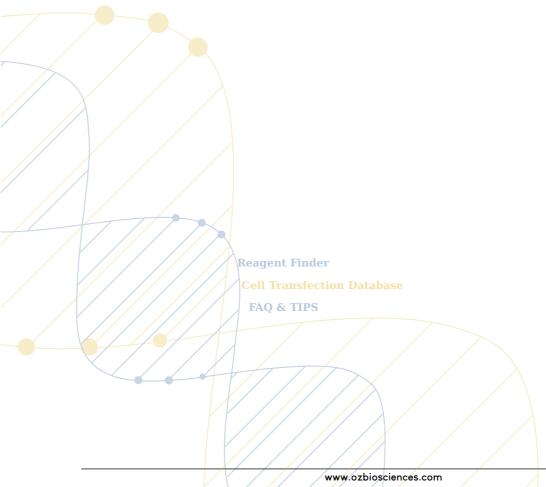
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## THCHNOLOGY DESCRIPTION

Magnetofection™ Technology

Polymer-based Transfection

Lipofection Technology

CRISPR/Cas9 Genome Editing

Mag4C Virus Concentration

i-MICST™ Technology

Transfection in 3D Cell Culture (see p.42)

*In vivo* Magnetofection™ (see p.54)

## MAGNETOFECTION™ TECHNOLOGY

Magnetofection™ is a simple and highly efficient transfection method. Inspired by the validated and recognized magnetic drug targeting technology, this original method is a revolution for transfection and infection. The idea was to **unite the advantages of the popular** biochemical (cationic lipids or polymers) and physical (electroporation, gene gun) **transfection methods in one system while excluding their inconveniences** (low efficiency, toxicity, difficulty to handle). **It is the sole technology suitable for viral and non viral gene delivery applications.** 

#### **PRINCIPLE**

Magnetofection  $^{\text{m}}$  principle is to associate nucleic acids, transfection reagents or virus with specific cationic magnetic nanoparticles. The resulting molecular complexes are then concentrated and transported into cells supported by an appropriate magnetic field. In this way, the exploitation of a magnetic force exerted upon gene vectors allows a very rapid concentration of the entire applied vector dose on cells, so that 100% of the cells get in contact with a significant vector dose, and promotes cellular uptake.

#### HOW DOES IT WORK?

The magnetic nanoparticles are made of iron oxyde, which is fully biodegradable, coated with specific proprietary cationic molecules varying upon applications. Their association with the gene vectors (DNA, siRNA, ODN, virus, etc.) is achieved by salt-induced colloidal aggregation and electrostatic interaction. The magnetic particles are then concentrated on cells by the influence of an external magnetic field generated by a specific magnetic plate. The cellular uptake of the genetic material is accomplished by endocytosis and pinocytosis, two natural biological processes.

Consequently, membrane architecture & structure stay intact in contrast to other physical transfection methods that damage, create hole or electroshock the cell membranes.

The nucleic acids are then released into the cytoplasm by different mechanisms depending upon the formulation used.

First is the proton sponge effect caused by **cationic polymers** coated on the nanoparticles that promotes endosome osmotic swelling, disruption of the endosomal membrane and intracellular release of DNA. Second is the destabilization of the endosome by **cationic lipids** coated on the particles that release the nucleic acid into cells by flip-flop of cell negative lipids and charged neutralization.

Third one is the **usual viral mechanism** when virus is used.



Watch our video online!

#### BIODISTRIBUTION OF MAGNETIC NANOPARTICLES

The biodegradable cationic magnetic nanoparticles are not toxic at the recommended doses and even higher. Gene vectors/magnetic nanoparticles complexes are internalized into cells after 10-15 minutes i.e. much faster than any other transfection method.

After 24, 48 or 72 hours, most of the particles are localized in the cytoplasm, in vacuoles (membranes surrounded structure into cells) and occasionally in the nucleus. In addition, magnetic nanoparticles do not influence cell functions.

#### WHAT ARE THE APPLICATIONS?

Magnetofection<sup>™</sup> is the only versatile and universal technology to transfect or transduce cells, adapted to *in vitro* or *in vivo* applications and all types of nucleic acids (DNA, siRNA, dsRNA, shRNA, mRNA, ODN...) as well as viruses.

Tailored reagents are available including the popular NeuroMag - specific for neurons transfection - and the whole range of ViroMag reagents, to enhance transduction or infection.

NON VIRAL APPLICATIONS - perfect for primary and hard-to-transfect adherent cells			
PolyMag Neo	Polymer-based nanoparticles for all nucleic acids transfection		
CombiMag	Improves the efficiency of any transfection reagent		
Magnetofectamine™ O2 Kit	For all nucleic acids - Association of CombiMag + MTX transfection reagent		
NeuroMag	For Neurons transfection		
Glial-Mag	For Glial cells transfection		
SilenceMag	For siRNA applications		
FluoMag	Fluorescent Magnetofection reagents		
VIRAL APPLICATIONS - ideal for	VIRAL APPLICATIONS - ideal for any cells including adherent and suspension cells		
ViroMag	For enhancing viral transduction efficiency (suitable for all viruses)		
ViroMag R/L	For Lentiviral and Retroviral transduction		
AdenoMag	For Adenoviral and AAV transduction		
Mag4C-LV/Mag4C-AD	For capturing and concentrating Lentiviruses and Adenoviruses		
Viro-MICST™	For capturing virus and infecting cells		
In vivo APPLICATIONS			
<i>In vivo</i> PolyMag & DogtorMag	For all nucleic acids		
In vivo ViroMag	For enhancing viral transduction efficiency		
In vivo SilenceMag	For siRNA applications		

Magnetofection  $^{\text{TM}}$  has been successfully tested on a broad range of cell lines, hard-to-transfect and primary cells. It is perfect for non-dividing or slowly dividing cells, meaning that **the genetic materials can go to the nucleus without cell division**. We have shown that combining magnetic nanoparticles to gene vectors of any kind results in a dramatic increase of uptake of these vectors and high transfection efficiency.

Check out our website:
Reagent Finder
Citation database

#### HOW DO I USE MAGNETOFECTION™ REAGENTS?

The protocol is a very straightforward and easy procedure:

- 1. Dilute nucleic acids or vectors in serum-free medium or buffer and add Magnetofection  $^{\scriptscriptstyle{\mathsf{TM}}}$  reagent
- 2. Incubate 20-30 min
- 3. Add these complexes directly to cells
- 4. Apply the magnetic field (place the culture plate on the magnetic plate)
- 5. Incubate  $5\mbox{-}20$  min, remove the magnetic plate and cultivate cells until assay



#### DO I NEED SPECIFIC EQUIPMENTS?

Magnetofection  $^{\text{TM}}$  technology requires appropriate magnetic fields that magnetize nanoparticles in solution, forms a very strong gradient to attract the nanoparticles and covers all the surface of the plate. To perform efficient transfection or infection, suitable magnetic nanoparticles formulations and magnetic field, are the only necessity. Therefore, three optimized magnetic plates with improved properties have been especially designed for Magnetofection  $^{\text{TM}}$ : the Super Magnetic Plate, the Magnetic Plate with 96 individual magnets and the Mega Magnetic Plate. Their special geometry and organization produce a strong magnetic field that is suitable for all cell culture dishes and supports. All Magnetofection  $^{\text{TM}}$  starting Kits from OZ Biosciences contain a magnetic plate and the reagents appropriate to your needs; it gives you a convenient solution to start your study.

#### **Magnetic plates**

- **>** Suitable for all Magnetofection<sup>™</sup> reagents
- > Suitable for all cell culture dishes and supports

The Super Magnetic Plate is suitable for all cell culture supports including:

- •384-, 96-, 48-, 24-, 12-, 6-well plates
- •35, 60, 90 & 100 mm dishes
- •T-25, T-75 and any other flasks
- •Any other cell culture support (slide, chamber slide, array, roller, etc.)

The Magnetic Plate with 96-magnets is designed for 96-well culture plates

The Mega Magnetic Plate can hold 4 culture dishes or plates at the same time

- ▶Can be easily cleaned and decontaminated with 70% ethanol
- ▶Can be used within incubators and with robots
- **∍**Can be used at room temperature, 37°C, +4°C, etc.
- **▶**Compatible with culture plates from most common suppliers
- ▶ Magnetic properties, distance between magnets and cells and incubation time have been optimized to efficiently concentrate nucleic acids or virus onto cells and to promote their internalization
- >Solid, completely reusable, it is a one-time buy



Super Magnetic Plate
Convenient for all cell culture supports
Catalog number: #MF10000



Magnetic Plate with 96 magnets
Adapted to 96-well plates
Catalog number: #MF10096



Mega Magnetic Plate
To hold 4 culture dishes at one time
Catalog number: #MF14000

#### In vivo magnets



*In vivo* Magnetofection  $^{\text{TM}}$  (description p.54) has been designed for *in vivo* targeted transfection and infection.

The only requirement for  $in\ vivo\ Magnetofection^{TM}$  is a small magnet specifically designed for this application. Several kinds of magnets are provided depending of your application.

OZ Biosciences in vivo magnets set: 1 extra small cylinder (ø 2 mm), 1 small cylinder (ø 5 mm), 1 cylinder (ø 10 mm) and 1 square (18x18 mm)

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## POLYMER-BASED TRANSFECTION

After the development of Lipofection (lipid-based transfection method) and Magnetofection<sup>TM</sup> (magnetic nanoparticles-based transfection method), OZ Biosciences revolutionizes Polyfection with the design and synthesis of a novel patented Cationic Hydroxylated Amphiphilic Multi-block Polymer (CHAMP). We created Helix-IN<sup>TM</sup>, a totally new transfection agent based on the CHAMP<sup>TM</sup> technology, to mark the separation from what is usually being done with classic transfection methods. Formed by three moieties, it combines and introduces three synergistic notions:

- The concept of "passing through the membranes barriers" due to its charge, pH sensitive and hydrophobic properties
- The idea of "**stealth transfection**" where DNA is protected, masked & supported all the way to its nuclear uptake
- The concept of **biocompatibility** due to biodegradable and cleavable moieties

This polymer-based transfection technology is an optimized delivery system that allows high efficiency with low cellular stress.

#### **PRINCIPLE**

This new bi-functional cationic biopolymer is made up of three moieties, bearing different characteristics and functions:

- The first binds and condenses DNA to an unprecedented level and facilitates cytosol delivery
- ▶ The second component is a pH responsive and cleavable linker that improves cellular delivery by favoring endosomal membrane destabilization
- ▶ The third moiety with an optimized molecular weight serves as a DNA shield and nuclear uptake facilitator

**Helix-IN**<sup>TM</sup>, our new bi-functional polymer-based transfection reagent differs from others virus-like vectors: DNA is hidden from the cell until its delivery to the nucleus.

#### **HOW DOES IT WORK?**

#### 1- PROTECTION AND SERUM STABILITY

The design of Helix-IN<sup>TM</sup> allows the positively charged polyplexes to be stable in solution and not to aggregate overtime. The structure, polyamine composition & grafting density of the CHAMP<sup>TM</sup> polymers were finely tuned and optimized to place the polyplexes at the exact interface where solubility is not affected overtime. Moreover, hydrophilic groups were arranged within the polymer to lower interactions with negatively charged serum proteins (albumin...) for a more efficient gene carrier definition.

Polyplexes remains intact and DNA is protected from degradation...

The positive DNA/polymer charge keeps DNA bound to polymer, playing a key role in protecting nucleic acid from degradation by serum enzyme (no DNA degradation is observed even when incubated in 50% fetal calf serum at 37°C for 24 hours).

#### 2 - CELLULAR UPTAKE

Cationic complexes bind to cell membrane mainly through electrostatic interactions (**figure 1 - 1**) and most polyplexes are taken up by the cell through endocytosis pathways. One of the most known routes of endocytosis is mediated by clathrin (**figure 1 - 2**). Once endocytosed, complexes are internalized in an early endosome where pH drops from 7.4 (cell surface) to 6.0 (lumen of endosome). The pH will drop to 5 as the endosome progresses to its late phase.

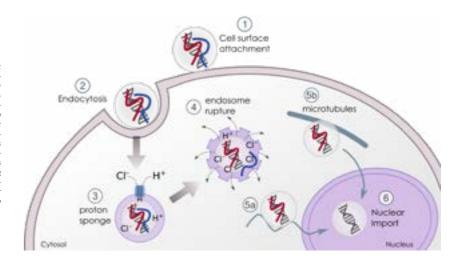
#### 3- ENDOSOMAL ESCAPE & DNA RELEASE

Polyplexes evade endosome and release their cargo into nucleus through the cationic polymer buffering capacities related to the "proton sponge" effect (**figure 1 - 3**). The massive and continue flow of protons is accompanied with passive entry of chloride ions that results in accumulation of water. As a consequence, the vesicles swell until endosomal rupture and their content is delivered into the cytosol (**figure 1 - 4**).

The pH responsive linker hidden at physiological pH gets exposed at acidic pH. This leads to its cleavage and to the hydrophobic zone exposition which promote endosomal membrane fusion/destabilization. At this stage, several important pitfalls can impair transfection efficiency:

- > The capacity of DNA to escape from endosomes is one of the major limitations of the transfection
- ▶ Once delivered into cytosol, DNA is usually must rapidly be imported in the nucleus to avoid cytosolic degradation
- ➤ Cell sensors in endosomes (also on cell surface) can recognize foreign nucleic acids and induce a protective response inhibiting transfection

Figure 1. Intracellular trafficking of polyplexes. Excess of polycations at the surface of the polyplex allows attachment to the cell surface (1) and uptake or internalization by the target cell, generally through endocytosis (2). Once into endosome vesicle (3), higher degree of protonation of the proton-sponge polymer causes influx of ions and the pH responsive linker is cleaved, releasing the first polymer unit (blue). The increase of osmotic pressure that leads the vesticle to swell and rupture is favored by the exposition of the hydrophobic domain of the linker (4). The third unit (red) remains bound to the nucleic acid thus lowering the sensing of the DNA by the cell and assisting its nuclear delivery through direct import into the nucleus (5a) or via microtubules trafficking (5b). Once inside the nucleus (6) the DNA is then expressed.



#### 4- TRANSPORT AND NUCLEAR INTERNALISATION

Once released from endosome, polyplexes have to migrate to the nucleus either via microtubules (figure 1 - 5a) or through nuclear import machinery (figure 1 - 5b). In general, large DNA molecules (>3000bp) and polyplexes remain almost immobile as diffusion is size-dependent into the cytoplasm and numerous cytosolic nucleases degrade nucleic acids. Being still complexed to the third moiety of our bi-functional polymer, the smaller positively charged polyplexes can interact with anionic microtubules or motor proteins, or diffuse in a stealth mode until their nuclear uptake. During all these procedures, the DNA is masked and protected from degradation.

#### WHAT ARE THE APPLICATIONS?

The principal use is **DNA transfection for** in vitro and in vivo applications.

The CHAMP<sup>TM</sup> technology increases transfection: more DNA enters the cells and DNA is addressed to the nucleus in a stealth mode without alerting and stressing the cell. Helix-IN<sup>TM</sup> is ideal for immortalized cell lines preferentially adherent such as HEK-293, NIH-3T3, CHO, COS-7, HeLa, MCF7, MEF, RPE-1, C2C12... **This reagent is perfect for co-transfection of multiple DNA.** 

For *in vivo* applications, the DNA is condensed and protected into small polyplexes that limit immune responses and are able to navigate through circulatory system until their delivery.

#### WHAT IS THE PROTOCOL?

The protocol is simple: transfection reagent is directly mix with DNA using ratios 1:1 to 3:1 (1 $\mu$ L per  $\mu$ g DNA to 3 $\mu$ L per  $\mu$ g DNA) depending on the cell type. After 30 min of incubation time, polyplexes and boost are added onto cells.

This 30 min incubation time is the cornerstone of the protocol allowing a full compaction and protection of DNA.





exes Add Boost

DNA 30 m incubat

Polyplexes

Add Boos and complexes to ce

During the nanoparticles/DNA complexes self-assembly, it is critical to wait at least 30 minutes to enable the co-polymers and DNA to form stable supramolecular nanoparticles. Due to the multipart nature of the copolymer, the time for forming and stabilizing the complexes is slightly longer than with "simple" polymers where complexes formation occurs more rapidly (10-20 min).

▶ To see difference between Lipofection and Polyfection please check out our website.

## LIPOFECTION TECHNOLOGY

#### **PRINCIPLE**

Lipofection is a lipid-based transfection technology which belongs to biochemical methods including also polymers, DEAE dextran and calcium phosphate. Lipofection principle is to associate nucleic acids with cationic lipid formulation. The resulting molecular complexes, known as lipoplexes, are then taken up by the cells. The main advantages of lipofection are its high efficiency, its ability to transfect all types of nucleic acids in a wide range of cell types, its ease of use, reproducibility and low toxicity. In addition this method is suitable for all transfection applications (transient, stable, co-transfection, reverse, sequential or multiple transfections...), high throughput screening assay and has also shown good efficiency in some in vivo models.

#### HOW DOES IT WORK?

#### **DNA Transfection Mechanisms**

The lipid-based reagents used for lipofection are generally composed of synthetic cationic lipids that are often mixed with helper lipids such as DOPE (L-a-dioleoyl-phosphatidylethanolamine) or cholesterol.

These lipids mixture assembles in liposomes or micelles with an overall positive charge at physiological pH and are able to form complexes (lipoplexes) with negatively charged nucleic acids through electrostatic interactions.

The association of the lipid-based transfection reagent with nucleic acids results in a tight compaction and protection of the nucleic acids and these cationic complexes are mainly internalized by endocytosis.

Once inside the cells two mechanisms leading to the nucleic acids release into the cytoplasm have been described. One relies on the endosomes buffering capacity of the polycationic residues (called "proton sponge effect"). The other describes the ability of cellular negatively charged lipids to neutralize the cationic residues of the transfection reagent leading to destabilization of endosomal membranes.

Finally, the cellular and molecular events leading to the nuclear uptake of DNA (not required for siRNA) following by gene expression remain highly speculative. However, the significance of cell division on transfection efficiency favours the assumption that nuclear membrane disruption during the mitosis process promote DNA nuclear uptake. Nonetheless, transfections of primary cells (non-dividing) and in vivo are also achievable with lipofection demonstrating that DNA can make its way to the nucleus where gene expression takes place.

#### Tee-technology

The cationic lipids (lipoplexes) and polymers (polyplexes) are the most employed non-viral gene delivery systems. The prioritary **Tee-technology** (Triggered Endosomal Escape) combines and exploits the properties of both entities to achieve extremely efficient nucleic acids delivery into cells.

Indeed, this new generation of patented lipopolyamines contains a lipophilic part, such as lipids, and a charged polyamine moiety, such as cationic polymers. These moieties act in synergy to ensure a tight nucleic acids compaction and protection and a very efficient destabilization of the endosomal membrane which allows the release of large nucleic acids amounts in the cytosol and DNA nuclear uptake. A particular focus on the synthesis of fully biodegradable entities was integrated. In this way, the transfection reagents do not interfere with cellular mechanisms, high cell viability is maintained in every experiment and any potential secondary effects are avoided.

#### WHAT ARE THE APPLICATIONS?

Transfection efficiency combined with high transgene expression level or high gene silencing and minimized cytotoxicity depends on multiple critical parameters. Those factors include cell type. plasmid DNA characteristics (size, promoter, reporter gene) & purity, siRNA sequence & purity, cell culture conditions (medium with or without serum, cell number, absence of contaminations...), amount of nucleic acids and reagents, transgene assays to name a few. Consequently, transfection reagents need to be specifically designed according to the nucleic acids to be delivered (DNA, siRNA, mRNA, ODN, shRNA, etc.) and the cell types used in order to achieve optimal efficiency. In this context, OZ Biosciences has developed several outstanding transfection reagents:

CLASSICAL TRANSFECTION	
DreamFect™ Gold	For all nucleic acids, acheving superior transgene expression level
DreamFect™/ DreamFect™ Stem	For all nucleic acids, for all cells including suspension cell lines
Lullaby <sup>®</sup> / Lullaby <sup>®</sup> Stem	For siRNA applications
RmesFect / RmesFect Stem	For mRNA applications
EcoTransfect	For popular cell lines and routine transfection at low cost
FlyFectIN™	For insect cells transfection
HeLaFect	For HeLa cells transfection
VeroFect	For Vero cells transfection
CosFect	For COS cells transfection
3D-TRANSFECTION	
3D-Fect <sup>™</sup> / 3D-FectIN <sup>™</sup>	For DNA transfection or gene silencing of cells growing in 3D-culture
BIOPRODUCTION	
HYPE transfection Kits	For Protein Production
HYVIR™ transfection reagents	For Virus Production
PROTEIN DELIVERY	
Ab-DeliverIN™	For intracellular antibody delivery
${\sf Pro-DeliverIN^{TM}/Pro-DeliverIN^{TM}CRISPR}$	For intracellular protein delivery

#### The major Tee-technology advantages are:

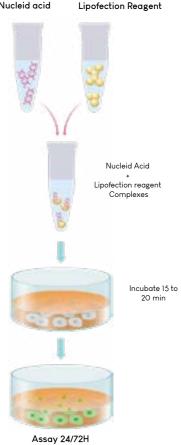
- Compaction of DNA in nanoparticles efficiently internalized by cells
- >Protection of nucleic acids against nucleases degradation >Efficient membrane destabilization and DNA delivery
- >Highly efficient even with low amounts of nucleic acids >Biodegradability



#### HOW DO I USE LIPOFECTION REAGENTS?

The protocol is a very straightforward and easy procedure:

- 1. Prepare the DNA and the reagent solutions
- 2. Mix them together and incubate 20 min
- 3. Add to your cells



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## CRISPR/CAS9 GENOME EDITING

"Genome editing" or "Genome engineering" gives the ability to introduce a variety of genetic alterations (deletion, insertion...) into mammalians cells. During the past decade, zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs) were the tools of choice for genome editing technologies until the recent discovery of CRISPR/Cas9 technology that have revolutionized the field.

Successful CRISPR/Cas9 genome editing can be performed through diverse approaches (plasmids, mRNA, nuclease, viral delivery). Accordingly, efficient nucleic acids delivery (transfection or transduction) represents a critical step for genome editing experiments. With more than 10 years of expertise in the development of transfection reagents, OZ Biosciences offers tailored transfection solutions for CRISPR/Cas9 technology:

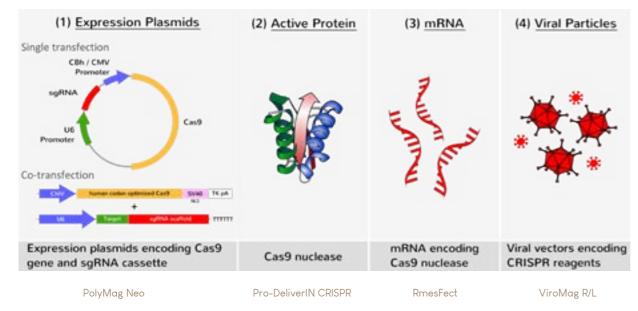


Figure 1: Adapted transfection reagents for each CRISPR/Cas9 approach. For generation of cellular models, Cas9 and the designed sgRNA (a chimeric RNA containing all essential crRNA and tracRNA components) can be introduced into the target cells. The type II CRISPR/Cas system only needs a single Cas protein that can be expressed into target cells by: (1) plasmid transfection, (2) direct delivery of the active Cas9 endonuclease, (3) transfection of mRNA encoding for Cas9 or (4) by viral vectors transduction.

#### Transfection Reagents For CRISPR/Cas9

Product Name	Molecul vector	Technology	Application
PolyMag Neo	Plasmid DNA	Magnetofection	Primary and hart-to-transfect cells
Pro-deliverIN CRISPR	Protein	Lipofection	All cells
RmesFect	mRNA	Lipofection	All cells
ViroMag R/L	Virus	Magnetofection	All cells including primary and hard-to-transfect cells

#### GENOME EDITING WITH CRISPR/CAS9

In 2013, four groups demonstrated that CRISPR/Cas9 associated with guide RNA can be used for gene editing. Based on the type II CRISPR/Cas9 mechanism, researchers created a single guide RNA (sgRNA), which is able to bind to a specific dsDNA sequence. This resulted in double strand breaks (DSB) at target site with: (1) a 20-bp sequence matching the protospacer of the guide RNA and (2) a protospacer-adjacent motif (PAM) 3 bp downstream NGG sequence. CRISPR/Cas9-mediated genome editing thus depends on the generation of DSB and subsequent cellular DNA repair process. The presence of DSB in the DNA generated by CRISPR/Cas9 leads to activation of cellular DNA repair processes, including non-homologous end-joining (NHEJ)-mediated error prone DNA repair and homology-directed repair (HDR)-mediated error-free DNA repair. Insertion and deletion mutations at target site generated by NHEJ and HDR allow disrupting or abolishing the function of a target gene. Moreover, modifications in this system can also be used to silence gene, insert new exogenous DNA or block RNA transcription.

#### HOW DOES CRISPR/CAS9 WORK?

CRISPR/Cas9 system originates from bacteria in which it provides acquired immunity against invading foreign DNA via RNA-guided cleavage. Bacteria collect "protospacers", short segments of foreign DNA (e.g. from bacteriophages) and integrate them into their genome. Sequences from CRISPR genomic loci are then transcribed into short CRISPR RNA (crRNA) that anneal transactivating crRNA (tracrRNAs) to destroy any DNA sequence matching the protospacers. After transcription and processing, crRNA first complexes with Cas9 and tracrRNA and then bind its target sequence onto DNA. Both R-loop forms and DNA strands are cut. crRNA is used as a guide while Cas9 acts as an endonuclease to cleave the DNA (figure 2).

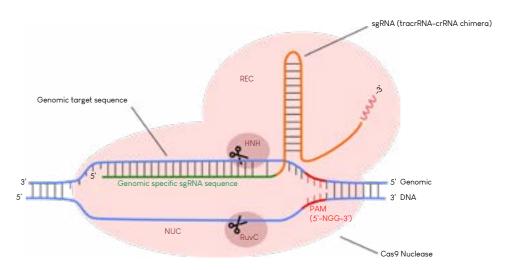


Figure 2: The CRISPR-Cas9 nuclease programmed with sgRNA. Upon binding the sgRNA guide (tracrRNA-crRNA) specifically targets a short DNA sequence-tag (PAM) and unzips DNA complementary to the sgRNA. sgRNA-target DNA heteroduplex, triggering R-loop formation results in a further structural rearrangement: Recognition (REC) and Nuclease lobes (NUC) undergo rotation to fully enclose the DNA target sequence. Two nuclease domains (RuvC, HNH) each nicking one DNA strand, generate a double-strand break. Structurally, REC domain interacts with the sgRNA, while NUC lobe drives interaction with the PAM and target DNA.

#### VARIOUS CAS9-BASED APPLICATIONS

- **→ Indel (insertion/deletion) mutations**
- > Specific sequence insertion or replacement
- **▶** Large deletion or genomic rearrangement (inversion or translocation)
- **>** Fusion to an activation domain:
- •Gene Activation
- •Other modifications (histone modification, DNA methylation, fluorescent protein)
- •Imaging location of genomic locus

#### CRISPR/CAS9 ADVANTAGES OVER ZFNs AND TALENS

CRISPR/Cas9 can easily be adapted to any genomic sequence by changing the 20-bp protospacer of the guide RNA; the Cas9 protein component remaining unchanged. This ease of use presents a main advantage over ZFNs and TALENs in generating genome-wide libraries or multiplexing guide RNA into the same cells.

- •ZFNs & TALENs are built on protein-guided DNA cleavage that needs complex protein engineering
- •CRISPR/Cas9 only needs a short guide RNA for DNA targeting
- •CRISPR/Cas9 allows using several gRNA with different target sites: simultaneously genomic modifications at multiple independent sites
- •Accelerates the generation of transgenic animals with multiple gene mutations

CRISPR/Cas9 system presents a versatile and reliable genome editing tool to facilitate a large variety of genome targeting applications. CRISPR/Cas9 components comprise an endonuclease and a sgRNA that can be delivered into cells under various forms (i.e. plasmid, mRNA, nuclease, virus).

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## MAG4C VIRUS CONCENTRATION

#### **PRINCIPLE**

**Mag4C magnetic nanoparticles** capture viruses in culture medium by electrostatic & hydrophobic interactions with 80-99 % efficiency. Once captured onto magnetic beads, viruses can be:

- ➤ Concentrated and stored with the Conservation Buffer or directly used for cell culture, molecular biology or other assays
- ➤ Concentrated, eluted from the magnetic beads with the Elution Buffer and stored with the Conservation Buffer or used for various assays



Watch our video online

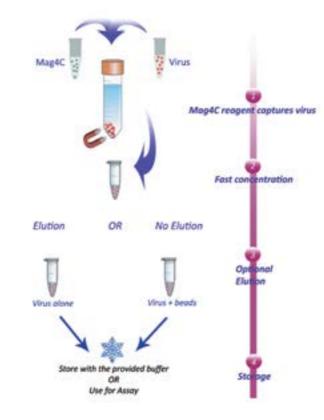
The two Mag4C-LV and Mag4C-AD kits are specifically designed and developed for capturing, concentrating and storing Lentiviruses/Retroviruses and Adenoviruses respectively.

These Kits are composed of 3 reagents allowing Magnetic Capture/Concentration, Elution and Conservation of viral particles and a multipurpose Magnetic Separation Rack.

The Conservation Buffer has been expressly designed to improve the stability of viral particles upon storage conditions. This buffer is fully compatible with magnetic nanoparticles, meaning that viruses bound to magnetic beads can be diluted directly into the buffer for long term storage.

#### WHAT ARE THE APPLICATIONS?

The magnetic action of the nanoparticles allows a **rapid concentration** of viral particles with minimized hazardous handling for a high yield of viral capture and recovery. The use of a magnetic field is simple, rapid and easy to use, and avoid time-consuming ultracentrifugation, precipitation and chemical steps.



#### WHAT CAN I DO WITH MAGNETICALLY CONCENTRATED VIRUS?

After magnetic capture and concentration, viruses can be used for multiple assays.

Viruses can be directly used with the bound Mag4C beads or eluted for *in vitro* and *in vivo* infection. Mag4C beads are compatible with the Magnetofection  $^{\text{\tiny TM}}$  technology.

This method allows concentrating the entire viral dose on the cells very rapidly, accelerating the transduction process and infecting non-permissive cells. Moreover, virus infection efficiency is significantly increased and cell adsorption/infection can be synchronized without modification of the viruses.

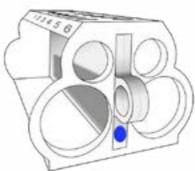
Targeted/confined transduction to specific area (magnetic targeting) can also be accomplished. Numerous applications were also demonstrated to be applicable to virus bound to magnetic beads, such as PCR, Western Blot, ELISA, *in vitro* and *in vivo* infection, etc.

The Elution Buffers offer in parallel the possibility to elute the viral particles from the magnetic beads. Buffers are specific from Adenovirus and Retro/Lentivirus as interactions engaged with the Mag4C beads are different. Once eluted, the magnetic nanoparticles are retained into the tube through the action of the magnetic field while eluted viruses are recovered. Elution buffers are totally compatible with virus viability and cell biology.

Once concentrated, the viral particles can be stored in the conservation buffer either bound to the magnetic nanoparticles or eluted. In this way, the stability of the viruses is improved under storage conditions. Conservation buffers are specific for Adenovirus or Retro/Lentivirus and totally compatible with virus viability and cell biology.

#### DO I NEED SPECIFIC EQUIPMENT?

Mag4C Kits need to be used with a Magnetic Separation Rack designed for 50, 15 or 1.5 mL tubes. It can hold 12 standard microtubes, two 15 mL and two 50 mL tubes. The Magnetic Separation Rack is required for capture, concentration, washing and elution when using Mag4C Kits.



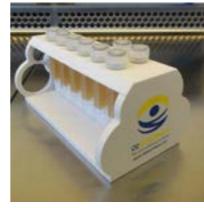
- •Can be easily cleaned and decontaminated with 70% ethanol
- •Can be used at room temperature, 37°C, +4°C, etc.
- Compatible with culture tubes from most common suppliers
- •Solid, completely reusable, it is a one-time buy
- •For centrifuge tubes only 15 mL: 17mm Ø

**OZ Biosciences MagID - Magnetic Isolation Device** is ideal for coupling reactions, washing procedures, aspiration, pipetting etc. MagID is made from an injection moulded plastic housing incorporating a high-energy neodymium magnet. It is designed to accommodate standard 1.5 mL tubes and is also suitable for some 2 mL tubes.

- •Ideal for your magnetic nanoparticles coupling reaction and purification
- •Adapted to working solutions ranging from 10 µL to 2 mL
- $\bullet$  Perfect for a quick magnetic separation process (< 5 minutes) with a high yield separation
- •Durable and easy-to-use with an open faced design



Magnetic separation rack can hold two 15 mL and two 50 mL tubes



Magnetic separation rack can hold 12 standard



MagID can hold 1 standard microtube

## I-MICSTM TECHNOLOGY



i-MICST™ technology (integrated Magnetic Immuno-Cell Sorting and Transfection/Transduction) is a new platform that allows to genetically modify cells directly on magnetic cell purification columns. This technology combines cell isolation and genetic modification in one simple, efficient and reliable integrated system. Designed for i-MICST™ technology, the Viro-MICST™ reagent allows the efficient and specific transduction of target cells directly on magnetic cell-purification columns.

#### WHY USE VIRO-MICST™?

Viro-MICST<sup>™</sup> leads to an increase in the transduction efficiency with low-titer virus preparations compared to regular transduction methods and allows you to reduce cell manipulation steps and save time as well as vector material.

- •Isolation and transduction of cells in one reliable integrated system
- High and increased transduction efficiency with low MOI
- •Acceleration of the transduction process and synchronization of adsorption
- •Ideal for sensitive cell types such as primary and stem cells

#### **HOW DOES IT WORK?**

i-MICST™ technology requires:

- **Magnetic cell separation systems** (not provided by OZ Biosciences)
- **The Viro-MICST™ reagent** for capturing virus and infecting cells within the magnetic cell purification column

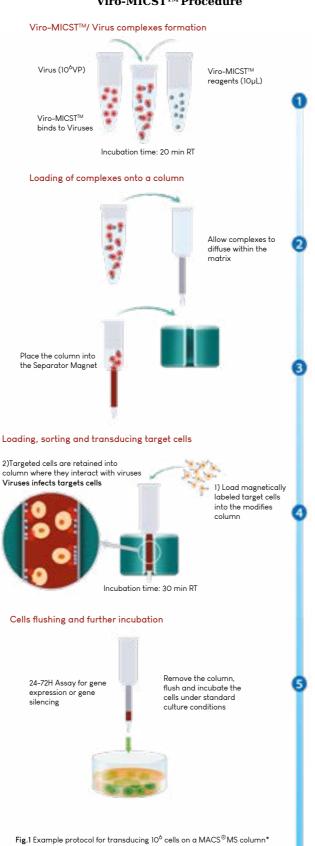
Viro-MICST $^{\text{TM}}$  is a new specific magnetic nanoparticle formulation evolved from our Magnetofection $^{\text{TM}}$  technology developed in association with MACS $^{\text{R}}$  technology\* from Miltenyi Biotec.

Viro-MICST $^{\text{\tiny TM}}$  binds to viruses. As both magnetically labeled target cells and virus-Viro-MICST $^{\text{\tiny TM}}$  complexes are retained by the magnetic field into the column, the viruses can interact and infect target cells with high efficiency.

The i-MICST $^{\text{\tiny TM}}$  protocol is depicted as a two-steps process:

- 1- Pre-enrichment step of magnetically labeled cells on non-modified column(s)
- 2- Viro-MICST™ procedure. This step allows reaching high purity and simultaneously infecting the target cell population (cf. fig.1)

## RAPID, SIMPLE AND READY-TO-USE Viro-MICST<sup>TM</sup> Procedure



For detailed information: www.ozbiosciences.com

\*MACS<sup>®</sup> is a registered trademark owned by Miltenyi Biotec GmbH and the use of MACS<sup>®</sup> column is proprietary and patented technology. For any further licensed of MACS<sup>®</sup> system, please contact Miltenyi Biotec.

www.ozbiosciences.com

## DNA TRANSFECTION

#### Cell Lines

Polyfection

Helix-IN™

Lipofection

 $\mathsf{DreamFect}^\mathsf{TM}\,\mathsf{Gold}$ 

EcoTransfect

#### **Primary Cells**

Magnetofection™

PolyMag Neo

CombiMag

LipoMag Kit

Magnetofectamine™ 02

#### **Cell Specific**

Magnetofection™

NeuroMag

Glial-Mag

#### Lipofection

HeLaFect

FlyFectin™

DreamFect™ Stem

#### Polyfection

VeroFect

#### **Explants**

XPMag Explant Transfection Reagent

 $Helix-IN^{\tiny{TM}}$  DNA transfection reagent based on  $CHAMP^{\tiny{TM}}$  technology opens up new possibilities for addressing issues of classical transfection technologies. This novel agent enables superior transfection performance, considering both the number of transfected cells and the yield of protein production. This bi-functional co-polymer is biocompatible, ionizable, pH responsive and biodegradable.

▶ To learn more about polymer-based transfection see page 12

#### **APPLICATIONS**

 Suitable for DNA transfections in immortalized cell lines: HEK-293, NIH-3T3, CHO, COS, HeLa...

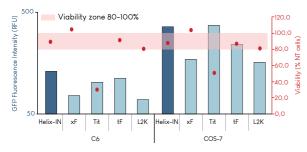


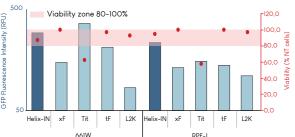
TRANSFECTION

Less DNA, Less Reagent, More Results



#### Helix-IN Outperforms Classical Transfection Reagents





High Intracellular Protein Production while Preserving Viability Various cell lines were transfected with Helix-IN and competitors according to their respective standard protocol. 48H after transfection, intracellular protein production was determined by cytofluorometry and cell viability was assessed on transfected cell monoloyers by MIT Assay (p.70 #MT01000).

#### PUBLICATION

22

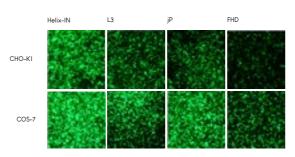
"Discover how to use Helix-IN to transfect prostate cancer cell line DU145."

HX11000

Liu X. et al - Nature Communications. 2018

▶ Browse our citation database online

## Transfection efficiency in classic cell lines with Helix-IN compared to competitors

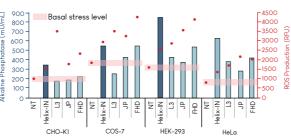


Cell lines were transfected with Helix-IN & competitors according to their respective standard protocol using pVectOZ-GFP. Transfection efficiency was monitored after 48H of incubation by fluorescence microscopy.

#### MAIN FEATURES

- Broad Spectrum DNA transfection reagent for cell lines and hard-to-transfect cells
- High transfection efficiency & transgene expression with low DNA amounts
- High intracellular protein production while preserving viability
- High secreted protein production while minimizing cellular stress
- Compatible with any culture medium
- Biodegradable
- Good for virus production
- ▶ For virus production see also Calcium Phosphate transfection Kit page 69

#### High Secreted Protein Production while Minimizing Cellular Stress



Various cell lines were transfected with Helix-IN and competitors according to their respective standard protocol. 48H after transfection, 25 µL of supernatants were analysed and cellular stress was evaluated on transfected cell monolayers using ROS Detection Assay Kit (p.70 #ROS0300).

## Cat. No. Product HX10100 Helix-IN 100 µL HX10500 Helix-IN 500 µl

No. of assays in a 24-well plate up to 100-200 up to 500-1000

Helix-IN 1 mL up to 1000-2000

Each kit contains 1 vial of Helix-IN reagent + 1 vial of HIB Enhancer reagent

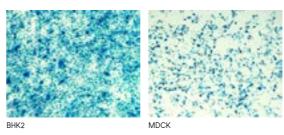
## $DreamFect^{\mathsf{TM}} Gold - \mathsf{Superior} \ \mathsf{delivery} \ \mathsf{of} \ \mathsf{nucleic} \ \mathsf{acids}$

DreamFect<sup>TM</sup> Gold, a lipid-based transfection reagent, allows transfecting all types of nucleic acids with a very high efficiency. Due to its formulation, this reagent delivers a large quantity of nucleic acids leading to higher protein expression compared to other transfection reagents. Consequently, high cell viability is maintained in every experiment and any potential secondary effect is avoided.

Depending on your cell lines (U-2-OS, HCT116, HA847, TM4, suspension cells...) we may recommend to use  $DreamFect^{TM}$ , the first version of DreamFect Gold.

▶ To learn more about Lipofection Technology see page 14

#### RFP-expression in different cell lines



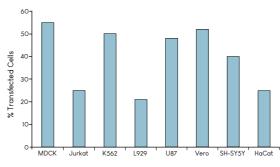
Different cell lines (1x10<sup>5</sup> cells/well) were transfected with 0.5 µg of pRFP plasmid DNA and 4 µL of DreamFect Gold reagent per well in a 24-well plate. RFF expression was monitored 24H after transfection by fluorescence microscopy.

#### MAIN FEATURES

- High protein expression level
   Ideal for biochemistry studies
- High & reproducible transfection efficiency Up to 95-99% transfection efficiency
- High compaction level of nucleic acids
- Highly efficient for a broad range of cells (3T6, A549, BEAS-2B, BHK-21, Jurkat...)
- Antibiotics & serum compatible
   Works over a broad range of cell confluencies (between 20 to 90%)
- Biodegradable

  Does not interfere with cellular mechanisms

#### DreamFect Gold™ Transfection Efficiency



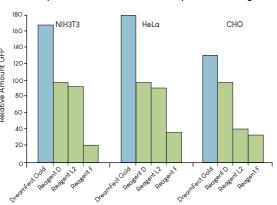
Several cell lines (Ix10<sup>5</sup> cells/well) were transfected with 1 µg of pEGFP plasmid DNA per well in a 24-well plate. Transfections were performed with 4 µL per well of DreamFect Gold transfection reagent. Percentage of transfected cells were measured 24H post transfection by cytofluorimetry

#### **APPLICATIONS**

- Suitable for all nucleic acids: DNA, oligonucleotides, mRNA, siRNA, shRNA...
- Perfect for all transfection applications in mammalian cells: co-transfection & reverse transfection, transient & stable transfection, High-Throughput Screening, etc.

# RECOMMENDED APPLICATION Transfection of cell lines with superior transgene expression level

#### Comparison of transfection efficiency with other reagents



#### **PUBLICATIONS**

"High transfection efficiency of HEK293T, MEF & NIH-3T3 with DreamFect Gold."
Infante P. et al - Nat Com. 2018

"MDCK stably transfected with DNA using DreamFect Gold."

Tlili S. et al - Royal Society Open Science. 2018

"COS and H661 cells transfected with DreamFect Gold"

O'Neill S. *et al* - **<u>IBC. 2018</u>** 

▶ Browse our citation database online

#### This product is also available in combination with CombiMag(p.26): LipoMag Kit

Cat. No.	Product	No. of transfections with 1 µg DNA
DG80500	DreamFect Gold 500 µL	125-250
DG81000	DreamFect Gold 1 mL	250-500
DG85000	DreamFect Gold 5x1 mL	1250-2500
The first version	of DreamFect Gold (ie DreamFect) is	still available:
DF40500	DreamFect 500 µL	125-250
DF41000	DreamFect 1 mL	250-500
DF45000	DreamFect 5 mL	1250-2500

## **EcoTransfect** - Economical transfection reagent for routine experiments

EcoTransfect is a lipofection reagent dedicated to the transfection of popular cell lines. This reagent is the perfect solution to quickly analyze the biological activity of your nucleic acids, to perform routine transfection assays at low cost and to accomplish High-Throughput Screening.

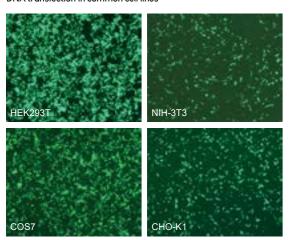
▶ To learn more about Lipofection Technology see page 14

#### **APPLICATIONS**

- Suitable for all nucleic acids delivery: DNA, oligonucleotides, siRNA...
- Perfect for all transfection applications in popular mammalian cells lines: transient or stable transfection, with or whitout serum...



#### DNA transfection in common cell lines



Cells (5 to 7.5x10<sup>4</sup> cells/well) were transfected with 1  $\mu$ g/well of pEGFP plasmid 8 2  $\mu$ L of EcoTransfect in 24-well plates. EGFP-expression was monitored 24H after transfection by fluorescence microscopy.

## PUBLICATIONS

24

"N1-Src Kinase is Required for Primary Neurogenesis in Xenopus tropicalis." COS-7

Lewis P.A. et al - Journal of Neuroscience. 2017

"COS-7 efficiently transfected with DNA using EcoTransfect."

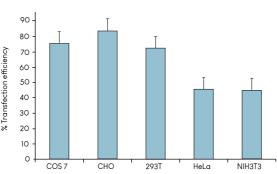
Keenan S. et al - Scienctific Reports. 2017

"HEK293 transfection with DNA using EcoTransfect"

Shepelev MV. et al - Mol Biol. 2018

▶ Browse our citation database online

#### EcoTransfect transfection efficiency in various cells



Cells (7.5x10<sup>4</sup> cells/well) were transfected in 24-well plates with 0.5 or 1 µg/well of pEGFP plasmid and 1 or 2 µl of EcoTransfect respectively, as described in the instruction manual. GFP-expression was monitored 24-48H after transfection by flow cytometry (FACS).

#### MAIN FEATURES

#### • The best quality/price ratio reagent

Provides identical performance than major reagents in most common cell lines at a very low cost

#### • Ideal for everyday experiments

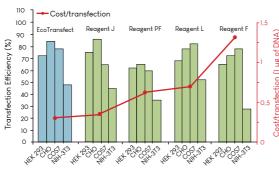
Ultimate solution to simply check biological activity of DNA constructs, insert (new clones), transcriptionally activated PCR fragments, mRNA or antisense oligonucleotides as well as producing stable transfection

#### Perfect for common cell lines 293, 293T, A293, CHO-K1, COS-1, COS-7, CV-1, HEK-293, HeLa, NIH-3T3...

#### • Biodegradable and non-toxic

#### • Serum compatible

#### Efficiency vs. Cost per transfection



Different cell lines (7.5x10<sup>4</sup> cells/well) were transfected with 1 µg/well of plas mid DNA (pEGFP) & 2 µL of EcoTransfect in 24-well plates. The others transfection reagents were assayed according to the manufacturer's instructions GFP-expression was monitored 24-48H after transfection by FACS.

Cat. No.	Product	No. of transfections with 1 µg DNA
ET10500	EcoTransfect 500 µL	250
ET11000	EcoTransfect 1 mL	500
ET13000	EcoTransfect 3x1 mL	1500

## PolyMag Neo - Ideal for primary & hard-to-transfect cells

PolyMag Neo is a polymer-based Magnetofection<sup>TM</sup> reagent specifically designed to achieve high transfection efficiency combined with superior transgene expression level. It is composed of magnetic nanoparticles coated with cationic molecules. PolyMag Neo is the ideal transfection reagent for a wide variety of cells. Depending on your application, we may recommend PolyMag, the first version of PolyMag Neo.

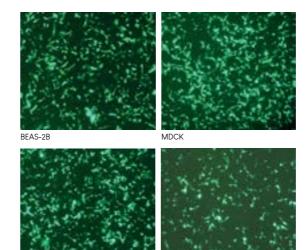
▶ To learn more about Magnetofection™ Technology see page 7

#### **APPLICATIONS**

- Suitable for all nucleic acids delivery: DNA, oligonucleotides, mRNA, siRNA, shRNA...
- Perfect for all transfections: transient & stable transfection, with or without serum...

# RECOMMENDED APPLICATION Transfection of primary and hard-to-transfect adherent cells

PolyMag Neo transfection efficiency in various cells



lxIO<sup>5</sup> cells were transfected with PolyMag Neo reagent in 24-well plates. EGFP-expression was monitored 24H after transfection by fluorescence microscopy.

#### PUBLICATIONS

"Transfection efficiency of N2A (neuroblastoma) was in the range of 70-80% using PolyMag Neo". Chou CC. et al- Nature Neuroscience. 2018

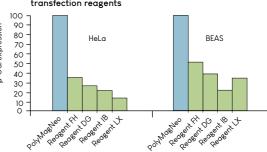
"Over expression of phosphatase and tensin homolog in primary human trabecular meshwork cells." Tellios N. et al - Scientific Reports. 2017

"Primary human neonatal cardiomyocytes successfully transfected with plasmid DNA using PolyMag". Bittel DC. et al-Cells. 2014

"DNA transfection, gene silencing and co transfection (DNA+siRNA) in HUVEC using PolyMag". Acosta MI. et al - Sci Rep. 2018

▶ Browse our citation database online

#### PolyMag Neo efficiency compared to classical transfection reagents

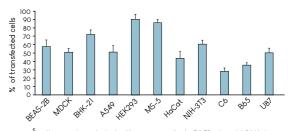




#### MAIN FEATURES

- Ideal for primary cells
   Epithelial, Fibroblast, Chondrocyte, Endothelial, (HUVEC, PAEC...), Stem Cells, etc.
- Powerful for hard-to-transfect cells 3Y1, AR42J, CT-26, Embryonic Stem Cells (D3ES), F9, FaDu, H441, HaCaT...
- High compaction level of nucleic acids
- High transgene expression & transfection efficiency
- Compatible with and without serum containing culture media
- Non-toxic

#### PolyMag Neo transfection efficiency in various cell lines



IxIO<sup>5</sup> cells were transfected with 0.5 µg/well of pEGFP plasmid DNA in 24-well plates. Transfections were performed with 0.5 µL/well of PolyMag Neo reagent. Percentage of transfected cells was measured 24H post transfection by flow cytometry.

For in vivo applications please refer to in vivo PolyMag page 55

#### This product is also available fluorescently-labelled with TRITC: FluoMag-P (#FP10100)



	Mindallerolection	rectificingy - this reagent needs to be used with a magnetic plate (p.9)
Cat. No.	Product	No. of transfections with 1 µg DNA
PG60100	PolyMag Neo 100 μL	100
PG60200	PolyMag Neo 200 μL	200
PG61000	PolyMag Neo 1 mL	1000
KC30200	Magnetofection Starting Kit	Contains 1 magnetic plate + 100 µL of PolyMag, PolyMag Neo, CombiMag
The first version of Poly	Mag Neo (ie PolyMag) is still availat	ole:
PN30100 / PN30200	PolyMag 100 μL / PolyMag 200 μL	100 / 200
PN31000	PolyMag 1 mL	1000

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TRANSFECTION

## OmbiMag – Boost all transfection reagents efficiency

CombiMag is the only existing reagent based on Magnetofection<sup>™</sup> for improving your transfection reagent efficiency. It can be used with any commercial transfection reagent and it has been successfully tested on a broad range of primary, hard-to-transfect cells and cell lines. CombiMag allows creating your own optimal delivery system with at least 30% transfection efficiency enhancement.

▶ To learn more about Magnetofection Technology see page 9

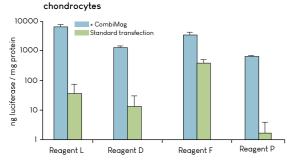
#### **APPLICATIONS**

TRANSFECTION

- Ideal for mammalian cells: cell lines, primary and hard-to-transfect cells
- Perfect for all transfection applications: transient & stable transfection, gene silencing...
- Suitable for all nucleic acids: DNA, oligonucleotides, mRNA, siRNA, shRNA...

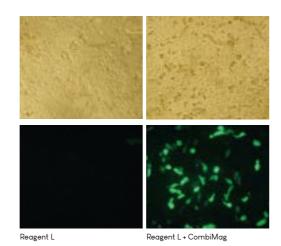
## RECOMMENDED APPLICATION Enhancing any transfection reagent efficiency for primary & hard-to-transfect cells

Luciferase expression in primary rabbit articular



Cells were transfected with various commercial reagents without or with CombiMag. We are grateful to Dr. U. Schillinger (Technical University, Munich) for kindly providing these data.

Transfection of primary keratinocytes using a commercial transfection reagent alone or in combination with CombiMag



#### MAIN FEATURES

- · Increase any transfection reagent efficiency
- · Save materials and time
- Ideal for primary & hard-to-transfect cells
- Highly efficient for cell lines: 3T6, A549, B16-F10, BEAS-2B, BHK-21, CV-1, L929, LoVo, N2A, U87...
- Simple, rapid and non-toxic

## PUBLICATIONS

"Mouse bone marrow macrophages transfection with DNA using CombiMag." Iwata H. et al- Nature Communications. 2016

"Efficient transfection method in MEF cells - in our analysis, Magnetofection (CombiMag), together with lipid reagents, resulted in a 3- to 13-fold increase in transfection efficiency compared with the lipid reagent alone."

Lee M. et al - J Biomol Tech. 2017

▶Browse our citation database online

For an optimized delivery system, use CombiMag in association with MTX reagent (p27):

Magnetofectamine O2

This product is also available fluorescently-labelled with TRITC: FluoMag-C (#FC10100)

Cat. No.	Product	No. of assays with 1 µg of DNA
CM20100	CombiMag 100 µL	100
CM20200	CombiMag 200 µL	200
CM21000	CombiMag 1 mL	1000
KC30200	Magnetofection Starting Kit	Contains 1 magnetic plate + 100 µL of PolyMag, PolyMag Neo, CombiMag

## <sup>™</sup>LipoMag Kit - Boosted transfection

LipoMag Transfection Kit associates our most efficient lipid-based transfection reagent, DreamFect Gold with CombiMag magnetic nanoparticles, the most versatile Magnetofection  $^{\text{m}}$  reagent. By taking advantage of the Lipofection and Magnetofection transfection techniques, LipoMag Kit reaches even higher transfection efficiency especially in primary cells while reducing toxicity.

#### Magnetofection Technology - This reagent needs to be used with a magnetic plate (p.9)

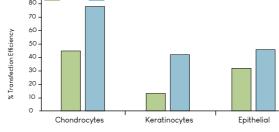
Cat. No.	Product	No. of assays with 1 µg of DNA
LM80250	LipoMag Kit : CombiMag 250µL + DreamFect Gold 500µL	100
LM80500	LipoMag Kit CombiMag 500µL + DreamFect Gold 1 mL	200
LM80000	Starting Kit: Super Magnetic Plate + 250µL of CombiMag, 500µL of DreamFect Gold	d 100

## Magnetofectamine MO2 - Ideal system for gene expression

Magnetofectamine™ O2 Kit has been designed for primary and hard-to-transfect cells. The alliance of MTX transfection reagent with CombiMag is the perfect one to lead to increased transfection efficiency, minimized toxicity and enhanced gene expression. CombiMag reagent binds to MTX transfection reagent/DNA complexes and under the application of a magnetic field concentrates the genetic material onto cells and promotes cellular uptake. In this way, transfection efficiency is enhanced.

▶ To learn more about Magnetofection Technology see page 9

# Magnetofectamine™ O2 Efficiency for primary cells 90 80 70 60



Various primary cells were transfected with LTX or Magnetofectamine™ O2 (MTX-O2). Results showed that Magnetofectamine O2 outperforms LTX transfection efficiency.

· Boost transfection efficiency with reduced

Low amount of nucleic acids - minimized

· No need to change your standard protocol

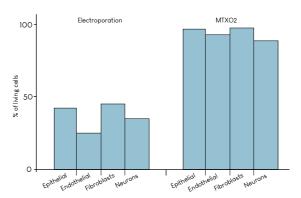
• Ideal for hard-to-transfect & primary cells

#### **APPLICATIONS**

- Perfect for all transfections: transient, stable, gene silencing, with or without serum...
- Suitable for all nucleic acids: DNA, oligonucleotides, mRNA, siRNA, shRNA...

# RECOMMENDED APPLICATION Transfection of primary and hard-to-transfect adherent cells

#### Comparison between Magnetofectamine O2 & Electroporation



Cytotoxicity comparison on primary cells between 2 transfection methods Electroporation and MTXO2

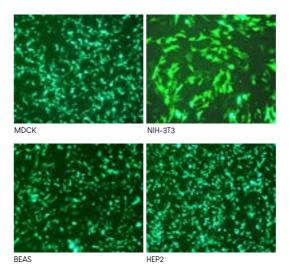
#### Magnetofectamine™ O2 Efficiency for cells lines

MAIN FEATURES

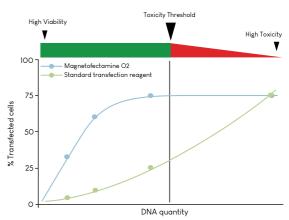
Serum compatible

cell toxicity

toxicity



#### Magnetofectamine O2 Viability and Efficiency



As the magnetic force drives the gene vector towards the target cells, Magnetofectamine<sup>TM</sup> O2 allows the vector dose to concentrate onto the cells very rapidly and triggers delivery via endocytosis. Consequently, high transfection efficiencies can be achieved with less nucleic acid amount.

Magnetofection Technology - This reagent needs to be used with a magnetic plate (p.9)

www.ozbiosciences.com

 Cat. No.
 Product

 MTX2-0750
 Magnetofectamine 250 µL +750 µL

 MTX2-1000
 Magnetofectamine Starting Kit

Description 250 µL CombiMag + 750 µL MTX reagent + 3 mL MTX Boost 100X 1 super magnetic plate (MF1000) + MTX2-0750

## NeuroMag - Powerful transfection reagent for neurons

Glial-Mag - The solution for Glial cells

NeuroMag transfection reagent has been designed for neurons transfection from 1 DIV to 21 DIV. It has proven to be extremely efficient in transfecting a large variety of primary neurons with all types of nucleic acids. Due to its unique properties, this reagent allows following the maturation of transfected neurons during several days after the transfection experiment.

▶ To learn more about Magnetofection Technology see page 9

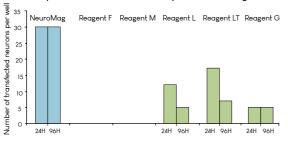
#### **APPLICATIONS**

TRANSFECTION

- Transfection of all types of nucleic acids: DNA, oligonucleotides, siRNA, mRNA, shRNA...
- · Suitable for all kinds of neural cells: Perfect for primary neurons: hippocampal, cortical, embryonic DRG, cerebellar granules, motorneurons, Neural Stem Cells... Neural cell lines: A172, B65, C6, KS-1, N2A, PC12, SH-SY5Y, SKN-BE2, T98G, U251, U87, YH-13...

## Successfully tested and published!

Comparison with other commercially transfection reagents



Rat hippocampal primary neurons were transfected after 15 DIV using NeuroMag or using competitor's transfection reagents according to the manufacturer's manuals. Transfection efficiency was monitored by fluorescence microscopy at 24H or 96H post-transfection.

#### **TESTIMONIALS**

"Due to its high efficiency and its low toxicity, we used NeuroMag to transfect cortical neurons." Charrier C. et al - Cell. 2012

"Transfection efficiency of primary cortical neurons was in the range of 20–30% for overexpression, and 10-15% for TDP-43 knockdown experiments Chou C.C. et al - Nature Neuroscience. 2018

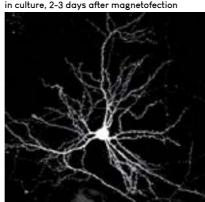
"Transfection of small RNAs (siRNAs, siPOOLs or sgRNAs) in primary Retinal Ganglion Cells using NeuroMag transfection reagent. " Welsbie D.S. et al - Neurons. 2017

"Use of NeuroMag to effectively transfect primary cortical neurons and iPSC-derived neurons " Wang W. et al - Nat Med. 2016

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#### RECOMMENDED APPLICATION Transfection of neuronal cells

Mouse cortical neuron expressing GFP (3 weeks

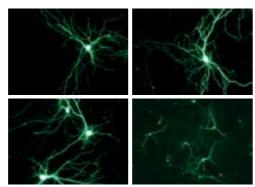


Results were kindly provided by Dr. C. Charrier (Charrie C. et al., 2012, Cell, Vol 149, Issue 4, 923-935).

#### MAIN FEATURES

- High efficiency from 1 DIV to 21 DIV
- · High transfected neurons viability
- Long transgene expression (up to 7 days)
- Non-toxic and completely biodegradable
- · Ready-to-use, straightforward and rapid

## Rat primary hippocampal neurons efficiently transfected



Primary rat hippocampal neurons were prepared in 24-well plates as after 14 DIV using 1  $\mu\text{g/well}$  of pEGFP plasmid and 3.5  $\mu\text{L}$  of NeuroMag Transfection efficiency was monitored by fluorescence microscopy 48h

#### Magnetofection Technology. This reagent needs to be used with a magnetic plate (n. 0)

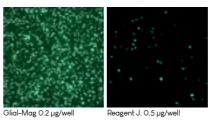
iviagnetorection Technology - This reagent needs to be used with a magnetic pi		
Cat. No.	Product	No. of transfections with 1 µg of DNA
NM50200	NeuroMag 200 µL	Up to 65
NM50500	NeuroMag 500 µL	Up to 165
NM51000	NeuroMag 1 mL	Up to 330
KC30800	NeuroMag Starting Kit	Contains 1 magnetic plate + 200 µL NeuroMag

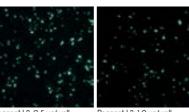
Glial-Mag Kit has been designed to reach optimal transfection efficiency with microglial cell lines and primary cells. This Koit is the association of a specific magnetic nanoparticles formulation (Glial-Mag reagent), issued from our Magnetofection™ technology and a booster (Glial-Boost) designed to enhance transfection efficiency.

▶ To learn more about Magnetofection Technology see page 9

#### RECOMMENDED APPLICATION Transfection of glial cells

Transfection of BV2 cells with Glial-Mag vs other commercial transfection reagents



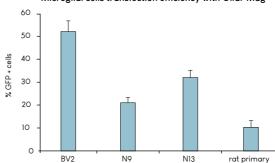


Complexes of pVectOZ-GFP encoding DNA and Glial-Mag were prepared as described in the standard protocol. Afte 24H, GFP+ cells were analysed by fluorescence microscopy.

#### MAIN FEATURES

- Highly efficient with microglial cell lines & primary cells
- · Low nucleic acid amount minimized toxicity
- High level of nucleic acid compaction
- Compatible with any culture medium

#### Microglial cells transfection efficiency with Glial-Mag

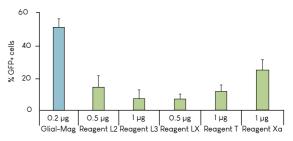


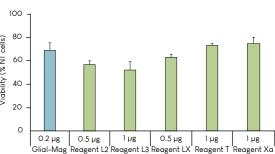
0.2 $\mu g$  of pVectOZ-GFP (BV2/Primary) and 0.4 $\mu g$  of pVectOZ-GFP (N9/N13) were complexed with Glial-Mag at a 3.5:1 ratio and transfection was performed according to the standard protocol. After 24H, GFP+ cells were analysed by flow cytometry

#### **APPLICATIONS**

- For transfection of microglial cell lines: BV2, N9, N13, HMO6, MG-5, SIM-A9, primary microglia
- Suitable for transient & stable transfection

Transfection Efficiency and Viability of BV2 cells with Glial-Mag compared to classical transfection reagents





Complexes of pVectOZ-GFP encoding DNA and Glial-Mag were prepared as described in the standard protocol. After 24H, GFP + cells were analysed by fluorescence microscopy and flow cytometry. Viability was assessed in parallel with the MTT cell proliferation Assay Kit (p.70 #MT01000) and compared to un-treated cells (NT).

#### **TESTIMONIAL / PUBLICATION**

"We are using the BV2 microglia cell line and have difficulties in transfecting those cells [...]. The transfection worked well with Glial-Mag and I did not observe cell death."

Math. C. - Karolinska Institutet - Stockholm -Sweden

"Magnetofection is superior to other chemical transfection methods in a microglial cell line." Smolders S. et al - J Neurosci Methods. 2018

"83-93% transfection efficiency of fluorescent siRNA in primary microglial cells and 60% in mRNA level decrease using Glial-Mag. No cell toxicity and inflammatory activation."

Carrillo-Jimenez A. et al - Frontiers in Cell Neuro.

▶Browse our citation database online

Magnetofection Technology - This reagent needs to be used with a magnetic plate (p.9)

Cat. No. Product GL00250 Glial-Mag 250 µL GL00500 Glial-Mag 500 µL KGL00250

250 µL of Glial-Mag reagent + 3mL Glial-Boost 500 µL Glial-Mag reagent + 6mL Glial-Boost

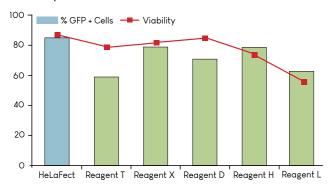
Glial-Mag Starting Kit 250 µL Glial-Mag reagent + 3mL Glial-Boost + 1 Super magnetic plate

TRANSFECTION

## HeLaFect Reagent - Optimized for HeLa Cells

HeLaFect is a lipid-based transfection reagent specifically developed for HeLa cell lineage transfection with high efficiency. Its design allows high nucleic acid compaction and leads to high viability. It is based on the Tee-Technology ("Triggered Endosomal Escape").

HeLafect transfection efficiency and viability compared to competitors



Complexes of DNA and HeLaFect were prepared with 0.5 µg per well in a 24-well plate at a 2:1 ratio, and DNA transfections with other transfection reagents were performed as recommended by the manufacturers. 24H after transfection efficiency was measured by FACS analysis and fluorescence microscopy.

#### PUBLICATION

"Development of a set of C•G-to-G•C transversion base editors from CRISPRi screens, target-library analysis, and machine learning"

Luke W Koblan. et al - Nat Biotechnol. 2021

#### ▶ Browse our citation database online

Cat. No.	Product
HF20500	HeLaFect 500 µl
HF21000	HeLaFect 1 mL
HF25000	HeLaFect 5 mL
111 23000	HOLDI CCC 5 THE

No. of transfections with 1 µg of DNA at 500 µL 125-250 at 1 mL 250-500 at 5 mL 1250-2500

## FlyFectin<sup>TM</sup> - Optimal Insect cells transfection

#### MAIN FEATURES

## • Very efficient and reproducible The complexes formed by DNA and

The complexes formed by DNA and FlyFectin $^{\text{TM}}$  allow highly efficient & reproducible transfection even with low amounts of nucleic acids

- Successfully tested on various insect cells Ag55, Anso, As43, Bm5, CI8, Cpp512, High5, IPBL-SF21, Kc167, Ld652, Mos20, S2, Sf9, SL-2, SL-3, SPC-SL52...
- Ideal for production of recombinant protein using Baculovirus expression system
- Serum compatible & non-toxic

## PUBLICATION

Highly efficient RepRNA delivery in C6/36 mosquito cells using Flyfectin.

"Mosquito cell-derived West Nile virus replicon particles mimic arbovirus inoculum and have reduced spread in mice."

Boylan B.T. et al - PLoS Negl Trop Dis. 2017

▶Browse our citation database online

 Cat. No.
 Product
 No. of transfections with 1 μg of DNA

 FF50500
 FlyFectin 500 μL
 125

 FF51000
 FlyFectin 1 mL
 250

 FF55000
 FlyFectin 5x1 mL
 500

RECOMMENDED APPLICATION

Nucleic acids transfection into

HeLa cells

#### MAIN FEATURES

- Highly efficient more than 80% of transfected HeLa cells
- · Ready-to-use: no need of additional buffer
- Low nucleic acid amount minimized toxicity
- High level of nucleic acid compaction
- Compatible with any culture medium

HeLafect transfection efficiency



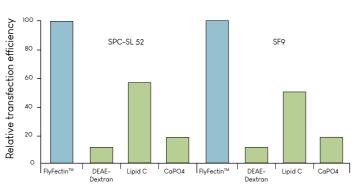




Z.

FlyFectin $^{\text{TM}}$  is a powerful reagent based on TEE-technology and specifically designed to obtain highly efficient and reproducible transfection of insect cells. It is adapted to all types of nucleic acids delivery and can be used for many applications including for the production of recombinant protein using Baculovirus expression system.

#### Comparison between FlyFectIN $^{\!\top\!\!M}$ & other transfection reagents



Cells were transfected according to the instruction manuals. Luciferase activities were measured with a Luciferase Assay Kit and results are expressed as relative values.

RECOMMENDED APPLICATION
Transfection of insect cells

# undifferentiated stage and their capacities to differentiate. To learn more about Lipofection Technology see page 14 APPLICATIONS Embryonic & Multipotent Stem Cells transfection

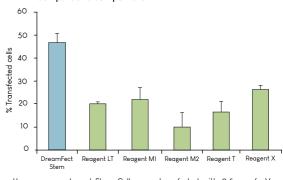
DreamFect™ Stem transfection reagent is a powerful reagent allowing multipotent Stem Cells trans-

fection with high efficiency & very low toxicity. Its specific composition based on the TEE-technology

allows transfecting embryonic and multipotent Stem Cells in presence of serum, maintaining their

DreamFect™ Stem transfection efficiency on MSC compared to competitors

 $DreamFect^{TM}\ Stem\ -\ {\tt DNA}\ delivery\ into}\ Stem\ {\tt Cells}$ 



Human mesenchymal Stem Cells were transfected with 0.5 µg of pVectOZ-GFP plasmid DNA and 1.5 µL of DreamFect™ Stem or with competitors' reagents (according to manufacturers' manuals). Transfection efficiency was monitored by flow cytometry 48H after transfection.

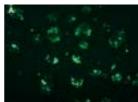
## $\mathsf{DreamFect}^\mathsf{TM}$ Stem allows high transfection rate of embryonic Stem Cells

• Suitable for all transfection applications:

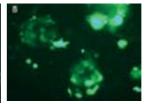
RECOMMENDED APPLICATION
Transfection of Stem Cells with high

transgene expression level

DNA transfection, High-Throughput Screening, Cell-based therapy, Regenerative medicine, Cell



reprogramming...

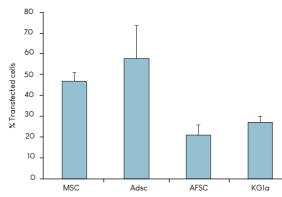


Mouse Embryonic Stem Cells growing on mitotically inactivated feeder cells were transfected with 1 µg of pVectOZ-GFP plasmid DNA and 3 µL of DreamFect™ Stem per well in a 24-well plate. Transfection efficiency was assessed by fluorescence microscopy 48H post transfection (Magnification x40 (A) and x 200 (B)).

#### MAIN FEATURES

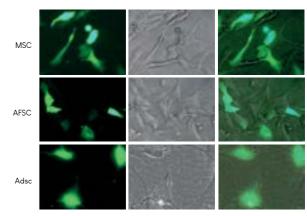
- High transfection efficiency for multipotent Stem Cells
- Minimized toxicity due to reagent biodegradability & low DNA amount required
- Cell phenotype and differentiation potential are not affected
- Serum compatible

#### DreamFect™ Stem transfection efficiency on different multipotent Stem Cells



Several human Stem Cells were transfected with 0.5 µg of pVectOZ-GFP plasmid DNA and 1.5 µL per well of DreamFect<sup>TM</sup> Stem reagent per well in a 24-well plate. Percentage of GFP positive cells were measured 48H post transfection by flow cytometry.

DreamFect™ Stem transfection efficiency on adherent Stem Cells



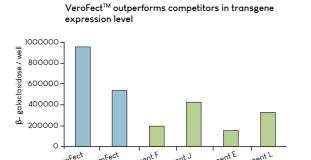
Adherent Stem Cells were transfected with 0.5 µg of pVectOZ-GFP plasmid DNA and 1,5 µL of Dreamfect<sup>™</sup> Stem per well in a 24-well plate. Transfection efficiency was assessed by fluorescence microscopy 48H post transfection.

Cat. No.	Product	No. of transfections with 1 µg of DNA
ST30500	DreamFect Stem 500 µL	125-250
ST31000	DreamFect Stem 1 mL	250-500
ST31000	•	250-500

## VeroFect - The solution for Vero Cells

VeroFect is a powerful polymer-based transfection reagent specifically designed to obtain highly efficient and reproducible transfection of Vero Cells. This reagent can be used for many applications such as stable and transient transfection, protein and viral production, etc.

▶ To learn more about Lipofection Technology see page 14



RECOMMENDED APPLICATION
Transfection of Vero cells

#### MAIN FEATURES

2500

- Higly efficient and reproducible

  The complexes formed by DNA and VeroFect
  reagent allow destabilizing cell membranes and
- Suitable for Vero & other immortalized related kidney cells

the delivery of important DNA amounts into cells

• Serum compatible & non-toxic

Cat. No.	Product
VF60250	VeroFect 250 μL
VF60500	VeroFect 500 µl
VF61000	VeroFect 1 mL
VF65000	VeroFect 5x1 mL

No. of transfections with 1 µg of DNA 125 250

## XPMag - Explant Transfection Reagent

XPMag is a novel magnetic nanoparticles formulation dedicated to gene transfection in organotypic cultures of explant by "Reverse Magnetofection". It provides a novel and non-toxic strategy for nucleic acids (DNA & siRNA) based gene therapy in the retina that can be translated to a wide variety of organ explants.

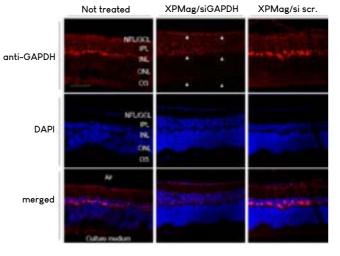
XPMag associated with Reverse Magnetofection allows the delivery of nucleic acids (NA) up to the deepest explant layers.

#### MAIN FEATURES

- Low nucleic acid amount minimized toxicity
- High level of nucleic acid compaction
- Easy and straightforward protocol
- Compatible with any type of explant



XPMag silences gene expression of GAPDH through the whole thickness of retina explants.



#### **PUBLICATION**

Effectiveve delivery of two drugs in mice's retina using XPMag.

"Magnetically Assisted Drug Delivery of Topical Eye Drops Maintains Retinal Function In Vivo in Mice." Bassetto M. et al - **Pharmaceutics 2021** 

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Magnetofection Technology - This reagent needs to be used with a magnetic plate (p.9)

Cat. No.	Product	No. of transfections with 1 µg of DNA	
XP00250	XPMag 250µL	125-150	
XP00500	XPMag 500µL	250-300	
KXP0250	XPMag Starting Kit	125-150	

## 3D TRANSFECTION

Technology Description

3D-Scaffolds

3D-Fect™ reagen

Hydrogels

3D-FectIN™ reagen

3D Transfection Protocol

(1) Preparation of complexes

(2) Incubation time: 20 min at RT

(5) Loading cells on :

(6) Assay (24/72H)

Gel polymerization (37°C x 30 min)

Complexes are embedded

in polymerized hydrogel

Cells are transfected

while invading get

(3) Complexes are added to:

3D Scaffold

3D Scaffold

Complexes are fixed

Cells are transfected while

colonizing 3D-scaffold

on scaffold surface

#### **PRINCIPLE**

Three-dimensional (3D) matrices, such as 3D-scaffolds and 3D-hydrogels, work as mechanical platforms for cell attachment and growth. Biomaterials, having a viscoelastic support in constant adaptation to external constraints and responding to numerous physiological stimuli, have been designed to mimic the organic milieu for cells1.

3D matrices allow cultivating cells in vitro in a more natural way. Therefore, 3D cell cultures assist the cell physiology analysis under conditions that more closely resemble to an in vivo-like environment compared to conventional 2D culture. Since last decade, it has been proposed that genetically modified cells growing on-, or embedded in 3D matrices could be used as a drug controlled release system2. Biomaterials for controlled delivery of plasmid DNA or siRNA can thus provide a fundamental tool to target transgene expression (over express or block) or can offer new perspectives for gene or cell therapy.

3D matrices can be composed by numerous materials (collagen, atelocollagen, polymers, hyaluronic acid, fibrin...) which are adapted to specific cell types. Consequently, to transfect cells on a variety of supports, OZ Biosciences has developed specific reagents.

#### **HOW DOES IT WORK?**

Based on a new technology, the 3D transfection reagents allow to genetically modify cells directly cultured in 3D environment with high efficiency. 3D transfection allows for a long term transgene expression (intracellular or secreted) or gene silencing. First, the nucleic acids (DNA, siRNA) are mixed with the 3D transfection reagent to form complexes. Then, those complexes are combined with the appropriate 3D matrices. Finally, the modified 3D matrices are colonized by cells to be transfected.

For More Infomation: "3D-fection: cell transfection within 3D scaffolds and hydrogels." Sapet C et al - Ther Deliv. 2013

#### WHAT ARE THE APPLICATIONS AND STUDIES?

Tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth, angiogenesis, tube and acini formation...3D matrices are routinely used in basic research and therapeutic applications. The 3D transfection reagents allow genetic modification of cells directly into or onto the matrices and thus in a more natural environment.

#### 3D-Fect<sup>™</sup> for 3D-Scaffolds

The 3D-Fect™ transfection reagent is specifically designed to bind and cover any kind of 3D scaffold.

#### 3D-FectIN<sup>™</sup> for Hydrogels

The 3D-FectIN™ transfection reagent is compatible with any hydrogel and allow transfecting cells directly cultured onto/into a hydrogel with high efficiency. It does not alter gelation or polymerization.

#### 1. Schmeichel KL, Bissell MJ. Modeling tissue-specific signaling and organ function in three dimensions. J Cell Sci 2003; 116: 2377-2388.

## 3D-Fect<sup>TM</sup> - A new outlook for your cells!

3D-Fect™ is a novel reagent, based on an innovative technology, specifically developed to directly transfect cells cultured in 3D scaffold. 3D matrices not only add a third dimension to cells environment, they also allow creating significant differences in cellular characteristics and behavior. In this way, scaffold-based 3D matrices combined with 3D-Fect/DNA complexes are colonized by cells to be transfected in a more natural environment.

▶ To learn more about Transfection in 3D cell culture see page 46

#### **APPLICATIONS**

• Perfect for all transfection applications in 3D scaffolds such as sponges, matrices, inserts: tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth....

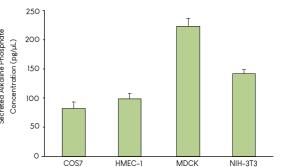
> RECOMMENDED APPLICATION DNA transfection of cells growing i 3D-scaffolds

#### MAIN FEATURES

- · Highly efficient on cell lines and primary cells
- Long term protein expression 3D-Fect<sup>™</sup> allows 3D trangene expression studies in in vivo like conditions over a long time period
- · Compatible with all types of nucleic acids
- · Gentle to cells

 $3D\text{-Fect}^{\text{TM}}$  is biodegradable & serum compatible and allows high cell viability

#### Alkaline phosphatase secretion of cells transfected into 3D Scaffold

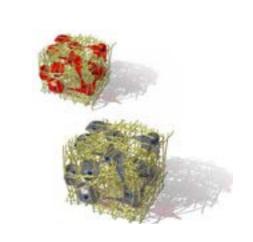


 $\mu g$  of pVectOZ-SEAP and 4  $\mu L$  of 3D-Fect reagent. Cells were then seeded and secreted alkaline phosphatase (SEAP) concentration was measured

#### Examples of 3D-Scaffolds successfully tested

Collagen-based Scaffold
Collagen-derived Scaffold
Hyaluronic Acid
Cell Culture Insert (Millipore)
Polycaprolactone
Poly(Ethylene Glycol)
Poly(Lactic-co-glycolic acid)
Poly(Styrene)
Poly(Urethane)

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Cat. No Product TF20250 3D-Fect 250 µL TF20500 3D-Fect 500 uL 3D-Fect 1 mL

No. of transfections with 1 µg DNA Up to 65 Up to 125 Up to 250

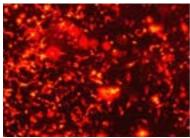
<sup>2.</sup> Scherer F et al. Nonviral vector loaded collagen sponges for sustained gene delivery in vitro and in vivo. J Gene Med 2002; 4: 634-643.

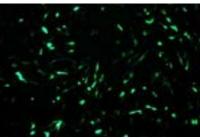
## 3D-FectIN<sup>TM</sup> - A novel perspective for your cells!

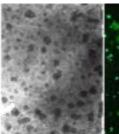
 $3D ext{-}\mathrm{FectIN^{ imes}}$  is the newest  $3D ext{-}\mathrm{transfect}$  in reagent specifically developed to directly transfect cells cultured in 3D hydrogels.  $3D ext{-}\mathrm{FectIN}$  is suitable for all kinds of hydrogels and cells. 3D matrices allow cells to grow in a micro-environment that more closely mimics the 3D environment encountered by cells  $in\ vivo$ . Thus, hydrogel-based 3D matrices combined with  $3D ext{-}\mathrm{FectIN/}DNA$  complexes allow cells to be directly transfected in more natural surroundings.

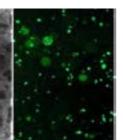
To learn more about Transfection in 3D cell culture see page 34

#### Transfection of various cells on different gels with 3D-FectIN





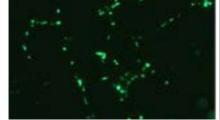


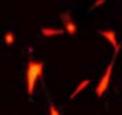


COS7-Collagen based hydrogel

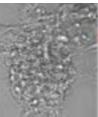
hMSC-Collagen derived hydroge

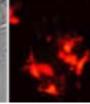
RAW-264,7 - HyStem-C











HMEC-1 - Matrigel™

Primary chondrocytes Collagen

Neural Stem cells - Matrigel™

#### MAIN FEATURES

Examples of 3D Hydrogels

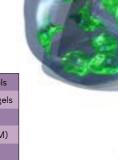
- Highly efficient on cell lines and primary cells
- Compatible with all types of nucleic acids
- Long term protein expression
- $\bullet$  Non-toxic and serum compatible

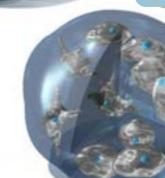
#### **APPLICATIONS**

- Perfect for all transfection applications in 3D hydrogels: angiogenesis, tube and acini formation, colonization, neurite growth, tissue engineering & regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation...
- Suitable for all types of nucleic acids including: plasmid DNA, linearized DNA, double stranded RNA, mRNA, oligonucleotides

#### RECOMMENDED APPLICATION

Transfection of cells growing in 3D-hydrogels





Collagen	Collagen-based Hydrogels	
Collagen-derived	Collagen-derived Hydrogels	
H.A	Hyaluronic Acid	
Gelatin	Extracellular Matrix (ECM)	
Fibrin/ Fibronecin	ECM	
Fibrinogen	ECM	
Laminim	EECM	
Matrigel™	BD Bioscience	
Poly-(Ethylene glycol)	PEGylated hydrogels	

▶ Browse our citation database online

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Cat. No. TN30250 TN30500

Product 3D-FectIN 250 µL 3D-FectIN 500 µL 3D-FectIN 1 ml No. of transfections with 1 µg DNA Up to 65 Up to 125 Up to 250

1 mL Up to 250

## RNA TRANSFECTION

siRNA Transfection

Lipofection

Lullaby $^\circ$ 

<sub>-</sub>ullaby® Stem

Magnetofection™

SilenceMa

mRNA Transfection

Lipofection

RmesFect<sup>TM</sup>

RmesFect<sup>TM</sup> Stem

## Lullaby® - A sweet song for gene silencing

Lullaby is the ideal siRNA transfection reagent for gene silencing. Relying on the TEE-technology, it has been successfully tested on numerous cell lines, reaching up to 90% gene silencing with high reproducibility and a very low toxicity. It protects siRNA from extracellular degradation and has an outstanding ability to destabilize cell membranes. It allows reproducible delivery of important siRNA amounts into the cytosol and high cell viability is maintained in each experiment.

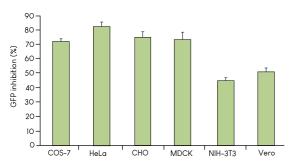
▶ Learn more about Lipofection Technology page 14

#### **APPLICATIONS**

- Perfect for all gene silencing applications: siRNA, shRNA, miRNA, dsRNA
- Suitable for all mammalian cells: cell lines, hard-to-transfect & primary cells

## RECOMMENDED APPLICATION siRNA transfection of cell lines. Perfect for High-Throughput Screening

GFP silencing in various cell lines with Lullaby



GFP-expressing cells were seeded on a 24-well plate and transfected with 10nM (67.5ng) siRNA associated with 2  $\mu$ L of Lullaby. GFP-extinction was monitored 72H post-transfection by flow cytometry.

#### **PUBLICATIONS**

"Development of a methodology for generating uniform and reproducible tumor spheroids that can be subjected to siRNA functional screening with Lullaby transfection reagent".

Utilizing Functional Genomics Screening to Identify Potentially Novel Drug Targets in Cancer Cell Spheroid Cultures

Morrison E. et al - Protocol JoVE

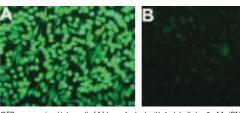
"Gene Silencing in MDA-MB-231 cells with siRNA using Lullaby".

Infante et al - Nature Communications. 2018

"Multiple sequential transfection of a large variety of cells with Lullaby siRNA transfection reagent". Jenks A.D. et al - Cell Reports. 2018

▶ Browse our citation database online

#### GFP silencing in HeLa cells



GFP-expressing HeLa cells (A) transfected with 1  $\mu$ L Lullaby 5 nM siRNA (B). GFP extinction was monitored 72H post-transfection.

#### MAIN FEATURES

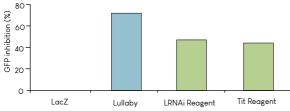
- Effective at multiple siRNA concentrations Minimize off-target effects
- Powerful for all cell types
  Up to 90% gene silencing 3T6, A549, BHK-21, CHO, COS-7, CV-1, H441, HEK-293, HeLa, M1...
- Flexible & adapted to all culture conditions
   Lullaby is antibiotics & serum compatible. It works
   over a broad range of cell confluencies (between
   20 to 90%)
- Versatile & convenient for all siRNA applications
   Tested on various RNAi targets (GAPDH, GFP, Kinase, LacZ, Lamin, Luciferase...) and with various synthetic siRNA & shRNA
- Rapid, easy procedure & biodegradable
- ▶ Working with Stem Cells? Lullaby Stem is ideal for gene silencing in Stem Cells, please refer to page 35

#### **TESTIMONIAL**

"We initially collated a transfection reagent library of 26 reagents [...]. By far, our prefered reagent is Lullaby. We have used this reagent in over 20 cell lines and have found it essential in enabling siRNA screens in hard to transfect cell lines [...], with minimal toxicity".

Shanks Emma.L. et al - Strategic siRNA Screening Approaches to Target Cancer at the Cancer Research UK Beaston Institute

GFP knockdown compared with classical reagents on A549 cells



GFP-expressing cells were seeded on a 24-well plate and transfected with 10nM (67.5ng) siRNA associated with 2µL of Lullaby. GFP-extinction was monitored 72H post-transfection by flow cytometry.

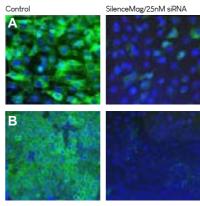
Cat. No. Product No. of assays
L170500 Lullaby 500 µL Up to 1000
LL71000 Lullaby 1 mL Up to 2000
LL73000 Lullaby 3x1 mL Up to 6000

SilenceMag – The bright idea for siRNA delivery

SilenceMag has been developed specifically for siRNA delivery. These magnetic nanoparticles are coated with a unique cationic lipids formulation providing the most efficient siRNA delivery system available. It allows studying gene silencing at very low doses of siRNA thanks to the magnetic field mediated concentration of siRNA onto cells. This reagent is suitable for all siRNA applications and gives reliable and high gene knockdown in numerous cell types.

▶ Learn more about Magnetofection Technology page 9

#### GFP silencing in HeLa cells



NIH-3T3 (A) and HEP2 (B) cells were treated with 5µL SilenceMag and 25nM siRNA targeting GAPDH gene

#### MAIN FEATURES

- High gene silencing efficiency Concentrates and introduces large quantities of siRNA into cells leading to exceptional knockdown effects
- Use 10 to 100 times less siRNA

  Gene silencing can be observed at 0.1 nM and efficiency is optimal at 5 to 10 nM
- One reagent validated for all siRNA applications

Effective for endogenous applications as well as co-transfection

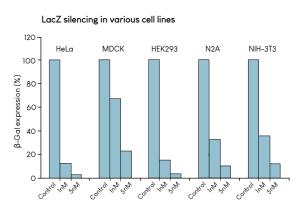
- Ideal for cell lines & primary cells Primary cells: Airway epithelial, Chondrocyte, Endothelial (PAEC, HUVEC...), Fibroblast, Gastric gland, Epithelial, Keratinocyte, Myofibroblast... Immortalized cells: BEAS-2B, CV-1, H441, Hep2, Hep3B, HMEC-1, MCF-7, MDCK, N2A, NIH-3T3, U87, Vero, etc.
- Serum compatible & non-toxic
- Many targeted genes

GAPDH, GFP, IGFBP, LacZ Lamin, Luciferase, Transcription factors, ROCK, etc.

#### **APPLICATIONS**

- Gene silencing: siRNA, dsRNA, shRNA
- Suitable for mammalian cells: cell lines, primary and hard to transfect cells

## RECOMMENDED APPLICATION siRNA transfection of primary & hard-to-transfect



Various cells were co-transfected in 96-well plates with 100ng of pLacZ plasmid complexed to PolyMag transfection reagents (p.23) and either 1 or 5 nM of siRNA associated with SilenceMag.



#### **PUBLICATIONS**

"68% of HUVEC were efficiently transfected". Dou L. et al - J Am Soc Nephrol. 2015

"90% gene silencing in primary human endothelial colony forming cells".

Hubert L. et al- J Thromb Haemost. 2014

"Gene Silencing in Endothelial Colony Forming Cells (ECFC) using magnetofection SilenceMag -Approximately 85-90% ECFC transfection efficiency was achieved".

Essaadi A. et al - Scientific Reports. 2018

"In vivo Gene Silencing of Endothelial cells using Magnetofection SilenceMag". Fujiu K. et al - Nature. 2017

▶Browse our citation database online

#### This product is also available fluorescently-labelled with TRITC: FluoMag-S (#FS10100)



Cat. No.	Product	No. of assays in 96-well plate with 10nM siRNA
SM10200	SilenceMag 200 μL	> 400 assays
SM10500	SilenceMag 500 µL	> 1000 assays
SM11000	SilenceMag 1 mL	> 2000 assays
SM13000	SilenceMag 3x1 mL	> 6000 assays
KC30300	SilenceMag Starting Kit	Contains 1 magnetic plate + 200 µL SilenceMag

## RmesFect<sup>TM</sup> - mRNA delivery solution

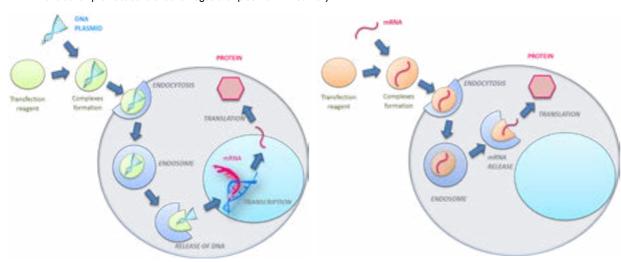
RmesFect transfection reagent, based on the TEE-technology, is specifically designed for mRNA transfection with high efficiency and low toxicity.

▶ To learn more about Lipofection Technology see page 14

TRANSFECTION

mRNA transfection provides two main advantages over plasmid DNA (pDNA) delivery. It does not require nuclear uptake for being expressed since translation of mRNA occurs into cytoplasm. Indeed, nuclear delivery (bypassing nuclear membrane) is one of the principal barriers for transfecting slow or non-dividing cells and consequently, mRNA transfection is particularly attractive for such purpose. Moreover, this approach presents also the advantage of not being integrative. Contrary to pDNA, mRNA cannot lead to genetic insertion causing mutations.

mRNA transfection provides several advantages over plasmid DNA delivery



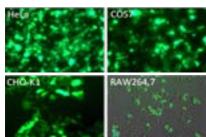
Transfection of mRNA with RmesFect holds several benefits:

- •No need for nuclear uptake mRNA translation into proteins occurs in the cytoplasm
- Faster protein expression than DNA transfection
- No genomic integration
- Protein expression in a total promoter-independent manner

#### **APPLICATIONS**

- · RmesFect transfection reagent is perfect for all your mRNA transfection applications:
- -mRNA vaccines/primary cells transfection
- -Regenerative medicine
- -Cell reprogramming
- -Embryonic & multipotent Stem Cells transfection

#### RECOMMENDED APPLICATIONS Transfection of mRNA in primary cells & cells lines



Several cell lines were transfected with mRNA encoding GFP protein using RmesFect transfection reagent. 24H after transfection efficiency was measured by fluorescence

RM20500

RM21000

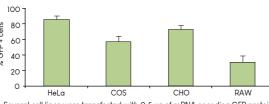
RM25000

Product RmesFect 500 ul

RmesFect 1 ml

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#### RmesFect™ transfection efficiency in cell lines



Several cell lines were transfected with 0.5 µg of mRNA encoding GFP protein (ratio 2:1 for RAW,3:1 for COS7 and CHO-K1 and 4:1 for HeLa cells). After 20 min of incubation at room temperature, complexes were added onto the cells in a dropwise manner. 24H after transfection efficiency was measured by FACS

#### MAIN FEATURES

- Ready-to-use No need for additional buffer
- Low nucleic acid amount
- Protects mRNA against degradation
- Compatible with any culture medium
- Serum compatible
- ▶ Working with Stem Cells? RmesFect Stem is ideal for mRNA transfection in Stem Cells, please refer to page 41.

No. of transfections with 1µg mRNA 125-250 250-500 1250-2500

Lullaby® Stem - Gene silencing in Stem Cells

Lullaby® Stem siRNA transfection reagent is ideal for gene silencing in Stem Cells.

▶ To learn more about Lipofection Technology see page 14





with 1µL Lullaby®Stem + 2nM siRNA trageting GFP.

GFP-stably transduced human AFSC (A) 48H after transfection

## **APPLICATIONS**

- Perfect for all gene silencing applications in Stem Cells: siRNA, shRNA, miRNA, dsRNA
- Suitable for all kinds of Stem Cells: Embryonic & Multipotent Stem Cells, iPS



Comparison of Lullaby Stem with other siRNA

#### MAIN FEATURES

- Minimized toxicity due to reagent biodegradability & low siRNA/miRNA amount required
- Reliable & reproducible gene knockdown results
- Serum & antibiotics compatible
- ▶ Browse our citation database online

LS20500

Lullaby Stem 500 µL

No. of assays Up to 250

LullabyStem

Reagent Iin

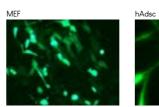
transfection reagents

80

60

## $\pmb{RmesFect}^{\mathsf{TM}} \; \pmb{Stem} \; \text{- mRNA delivery into Stem Cells}$

RmesFect Stem transfection reagent is based on the TEE-technology and specifically designed for mRNA transfection in Stem Cells with high efficiency and low toxicity.



No need for additional buffer

• Protects mRNA against degradation

• Compatible with any culture medium

• Low nucleic acid amount Minimized toxicity

• Serum compatible

MAIN FEATURES

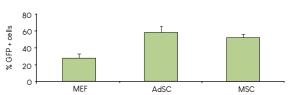
· Ready-to-use

Several Stem cells were transfected using RmesFect Stem, 24H afte

**APPLICATIONS** 

• Ideal for mRNA transfection applications in Stem Cells: mRNA vaccines, Embryonic & Multipotent Stem Cells transfection, regenerative medicine, iPS

> RECOMMENDED APPLICATION Transfection of mRNA in Stem Cells



Several Stem cells were transfected using RmesFect Stem. Complexes were prepared as followed: mRNA encoding GFP protein (0.25 µg for human hMSC and 0.5 µg for MEF and hAdSC) was mixed with RmesFect Stem. After 20 min of incubation at room temperature, the complexes were added onto the cells in a dropwise manner. 24H after transfection efficiency was measured

Cat. No.	Product	No. of transfections with 1µg mRNA	
RS30500	RmesFect Stem 500 µL	125-250	
RS31000	RmesFect Stem 1 mL	250-500	
RS35000	RmesFect Stem 5 mL	1250-2500	

## NOTES



# Reagent Finder Cell Transfection Database FAQ & TIPS

## BIOPRODUCTION

## Large-Scale Protein Production

HYPE-293 transfection Kit

HYPE-CHO transfection Kit

HYPE-5 transfection Kit

HYVIR™ transfection reagent

#### HYPE TRANSFECTION KIT

#### **Reach Optimal Protein and Antibody Production Yield**

As the development of stably-expressing cell lines can be laborious and challenging, transient transfection in mammalian cells has become the go-to method for obtaining milligram to gram quantities of recombinant proteins in a matter of days.

A key to a successful TGE (Transient Gene Expression) system and efficient protein production is to use a specific and appropriate transfection reagent.

At OZ Biosciences, we have developed two alternative and efficient transfection Kits, specific to HEK-293 and CHO cells, the two widely used hosts for TGE.

#### **HYPE-293**

HYPE-CHO

High Yield Protein Expression Designed for maximum efficiency in **HEK-293** cells growing in suspension

High Yield Protein Expression Designed for maximum efficiency in all **CHO-S** cells

#### HYPE-5

High Yield Protein Expression Designed for maximum efficiency in both HEK-293 and CHO cells

#### OVERCOMING BIOPRODUCTION CHALLENGES

HYPE transfection Kits allow high-level production of secreted or intracellular recombinant proteins for therapeutic or structural studies while overcoming the traditional challenges in bioproduction. These solutions are free of animal-origin components, an important quality for biotherapeutic applications.



REACH optimal Protein & Antibody production yield in suspension CHO & 293 cells

**OBTAIN** reproducible protein expression at various scales with minimal optimization

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**ENJOY** compatibility with multiple media formulations

**MEET** the quality requirements



## HYPE-293 Transfection Kit

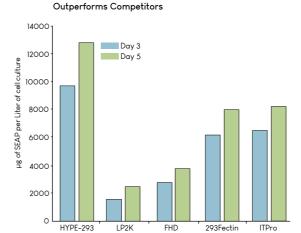
High Production Yield for Antibody

HYPE-293 transfection Kit is dedicated to achieve High Yield Protein Expression in HEK-293, 293-S, 293-F or any 293-related cells growing in suspension. Scale-up to larger volumes for production of milligrams of protein per liter of cell culture is straightforward and easy with simple and cost efficient handling steps.

#### **APPLICATIONS**

- Dedicated to protein production with **HEK293** cells in suspension
- Suitable for large-scale transient transfection: Ideal for bioreactor, spinner or flasks

#### RECOMMENDED APPLICATION Bioproduction and biopharmaceutical manufacturing



## 600 500 400 © 300 200 100

IgG Rabbit production after transient transfection of 293-F cells with HYPE-293 kit in 30 mL cell culture volume.

#### MAIN FEATURES

#### • High protein & antibody yield

Hype-293 transfection Kit achieves the highest protein yield in comparaison to other commercially available transfection reagents

#### • Easy & efficient scale-up

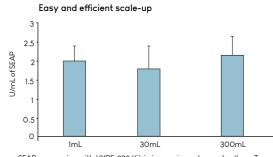
Protein production in 293-F suspension cells can be easily scaled up by using HYPE-293 transfection Kit without any protocol modification and protein yield production is even higher when compared to lower cell culture volume

- Suitable for all HEK-293 cells growing in suspension
- Compatible with any synthetic or regular media used for protein production
- Animal origin free
- If you work with CHO cells, please refer to HYPE-CHO transfection kit page 40.

#### **BROAD-SPECTRUM MEDIA COMPATIBILITY**

SEAP-expression with HYPE-293 Kit vs. other reagents

Medium	HYPE-293™
FreeStyle™ 293	✓
Expi293™	✓
EX-CELL® 293	✓
Pro293 <sup>™</sup> -CD	✓



SEAP-expression with HYPE-293 Kit in increasing volume of culture. Trans-Tections were performed using 0.75 or 1.5 µg of plasmid DNA per mL of cell culture. Ratio of 2 µL of HYPE-293 reagent per µg of plasmid was used. Density of cell culture was 1x10° cells/mL the day of transfection.

Additional Product: HYPE-5 transfection Kit - This generic Kit has been designed for recombinant protein expression in both HEK-293 and CHO cells growing in suspension. Cat. No.HY01500 (Hype-5 reagent 1.5 mL + Hype-5 Blast 5 mL)

Cat. No.	Product	Description	No. of transfections
HY29315	HYPE-293 Kit 1.5 mL	1.5 mL HYPE-293 + 5 mL B293	Suitable for 0.5-1 L of cell cultur
HY29330	HYPE-293 Kit 3 mL	2 x 1.5 mL HYPE-293 + 2 x 5 mL B293	Suitable for 1-2 L of cell culture
HY293150	HYPE-293 Kit 15 mL	15 mL HYPE-293 + 50 mL B293	Suitable for 5-10 L of cell culture
HY293300	HYPE-293 Kit 30 mL	2 x 15 mL HYPE-293 + 2 x 50 mL B293	Suitable for 10-20 L of cell cultu

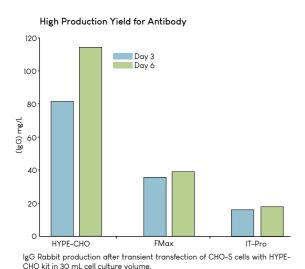
## **HYPE-CHO Transfection Kit**

HYPE-CHO transfection Kit has been designed for large scale up transient transfection and high protein expression such as antibody. The system is optimized for maximum efficiency in all CHO-S cells. It has been used and validated with cells from different origins (CHO, CHO-S, rCHO or any CHO-related cells) cultured in suspension (flask, spinner and bioreactor) used to produce proteins.

#### **APPLICATIONS**

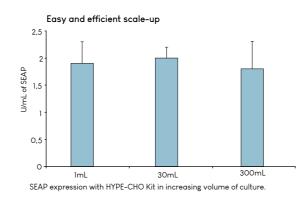
- Dedicated to protein production with CHO cells in suspension
- Suitable for large-scale transient transfection: Ideal for bioreactor, spinner or flasks





#### **BROAD-SPECTRUM MEDIA COMPATIBILITY**

Medium	HYPE-CHO™
FreeStyle™ CHO	✓
ExpiCHO™	✓
EX-CELL® CHO	✓
ProCHO™-CD	✓

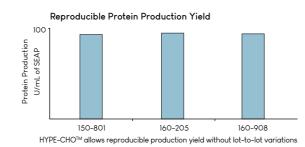


#### MAIN FEATURES

- High protein & antibody production yield HYPE-CHO transfection Kit achieves high antibody production level and largely outperforms other commercially avaible transfection reagents
- Easy & efficient scale-up

Protein production in CHO-S suspension cells can be easily scaled up by using HYPE-CHO transfection Kit without any protocol modification and protein yield production is even higher when compared to lower cell culture volume

- Suitable for all CHO cells growing in suspension
- Compatible with any synthetic or regular media used for protein production. HYPE-CHO Kit is highly efficient for intracellular protein production on cells adapted in suspension with chemically defined medium and in abscence of
- Animal origin free
- If you work with any 293 related cells, please refer to HYPE-293 transfection kit page 39.



Additional Product: HYPE-5 transfection Kit - This generic Kit has been designed for recombinant protein expression in both HEK-293 and CHO cells growing in suspension. Cat. No.HYO1500 ( Hype-5 reagent 1.5 mL + Hype-5 Blast 5 mL)

Cat. No.	Product	Description	No. of transfections
HYC01500	HYPE-CHO Kit 1.5 mL	1.5 mL HYPE-CHO + 5 mL BCHO	Suitable for 0.5-1 L of cell culture
HYC03000	HYPE-CHO Kit 3 mL	2 x 1.5 mL HYPE-CHO + 2 x 5 mL BCHO	Suitable for 1-2 L of cell culture
HYC15000	HYPE-CHO Kit 15 mL	15 mL HYPE-CHO + 50 mL BCHO	Suitable for 5-10 L of cell culture
HYC30000	HYPE-CHO Kit 30 mL	2 x 15 mL HYPE-CHO + 2 x 50 mL BCHO	Suitable for 10-20 L of cell culture

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and HYPE-Blast reagent (improves protein production in CHO cells).

• Dedicated to protein production with HEK293 and CHO cells in suspension

**APPLICATIONS** 

• Suitable for large-scale transient transfection: Ideal for bioreactor, spinner or flasks

#### RECOMMENDED APPLICATION Bioproduction and biopharmaceutica manufacturing

#### MAIN FEATURES

growing in suspension. It is composed of the HYPE-5™ transfection reagent (a Lipofection reagent)

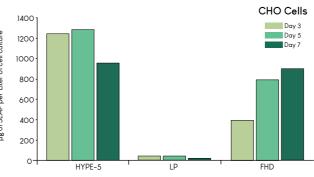
#### • High protein & antibody yield

Hype-5<sup>™</sup> transfection Kit achieves the highest protein yield in comparaison to other commercially available transfection reagents

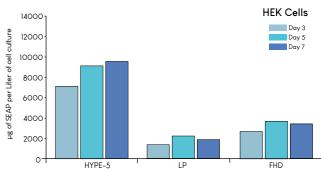
#### • Easy & efficient scale-up

Protein production in 293-F and CHO suspension cells can be easily scaled up by using HYPE-5™ transfection Kit without any protocol modification and protein yield production is even higher when compared to lower cell culture volume

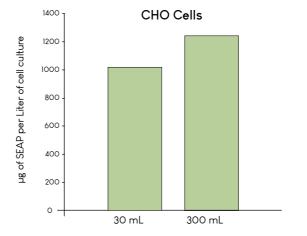
- Suitable for both HEK293 and CHO cells growing in suspension
- Compatible with any synthetic or regular media used for protein production
- Animal origin free

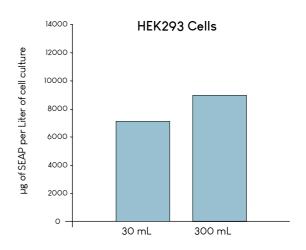


SEAP Expression in CHO cells with HYPE-5 Kit vs. other reagents.



SEAP Expression in HEK cells with HYPE-5 Kit vs. other reagents.





SEAP Expression in CHO and HEK cells with HYPE-5 Kit in increasing volume of culture.

#### Additional Product: HYPE-5 reagent- available in 3mL, 15mL and 30mL (Cat. No. HYR10003, HYR10015, HYR20030).

Cat. No.	Product	Description	No. of transfections
HY01500	HYPE-5 Kit 1.5 mL	1.5 mL HYPE-5 + 5 mL HYPE-5 Blast	Suitable for 0.5-1 L of cell culture
HY03000	HYPE-5 Kit 3 mL	2 x 1.5 mL HYPE-5 + 2 x 5 mL HYPE-5 Blast	Suitable for 1-2 L of cell culture
HY15000	HYPE-5 Kit 15 mL	15 mL HYPE-5 + 50 mL HYPE-5 Blast	Suitable for 5-10 L of cell culture
HY30000	HYPE-5 Kit 30 mL	2 x 15 mL HYPE-5 + 2 x 50 mL HYPE-5 Blast	Suitable for 10-20 L of cell cultu

## HYVIRTM Transfection Reagent - Virus Production

**HYVIR transfection reagent** is dedicated to achieve **H**igh **Y**ield **Virus production** in HEK-293 T. Its specific formulation allows an optimal plasmid compaction, protection and delivery rendering HYVIR ideal for the production of last generation of lentivirus.

#### **APPLICATIONS**

- Dedicated to enhance viral particles production in HEK-293 cells.
- Ideal for **last generation lentivirus**

# RECOMMENDED APPLICATION Lentivirus production

#### MAIN FEATURES

#### Performant

HYVIR reaches high titers functional lentiviral / virus production

#### • Highly efficient

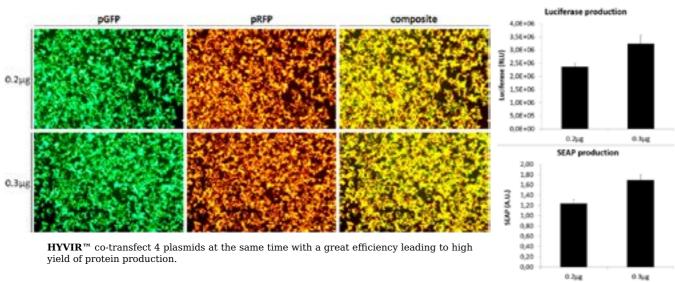
Co-transfection of 4 plasmids at the same time

#### • Reliable and reproductible

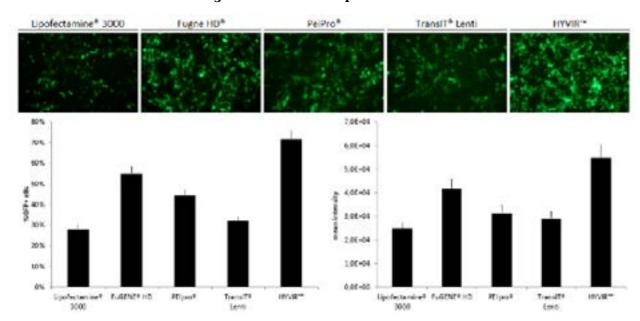
Easily scalable for production in large volumes and can be raised to superior grade

• Completely animal origin free

#### **Co-Transfection of 4 Plasmids**



#### **High Yield Lentivirus production**



 $Compared \ to \ competitors, \ \textbf{HYVIR}^{\text{TM}} \ induces \ the \ production \ of \ higher \ yields \ of \ infectious \ lentiviral \ particles.$ 

 Cat. No.
 Product

 HYV01500
 HYVIR Kit 1.5 mL

 HYV03000
 HYVIR Kit 3 mL

 HYV15000
 HYVIR Kit 15 mL

 HYV30000
 HYVIR Kit 30 mL

Description
HYVIR Reagent 1.5mL + HYVIR Boost 5mL
HYVIR: 2 x (1.5mL Reagent + 5mL Boost)
HYVIR Reagent 15mL + HYVIR Boost 50mL
HYVIR: 2 x (15mL Reagent + 50mL Boost)

No. of transfections
Suitable for 0.5–1 L of cell culture
Suitable for 1–2 L of cell culture
Suitable for 5–10 L of cell culture
Suitable for 10–20 L of cell culture

## IN VIVO TRANSFECTION

Technology Description

In vivo Magnetofection™

In vivo Transfection

In vivo DogtorMa

In vivo PolvMas

In vivo Infection

In vivo Viroma

In vivo Gene Silencing

In vivo Silence Mas

In vivo polymer-based Delivery System

Gene delivery in Central Nervous System

BrainFectIN™

#### IN VIVO MAGNETOFECTION™ TECHNOLOGY.

The main problems currently associated with systemic gene vector administration (gene therapy) include biodistribution of gene vector throughout the body, the lack of specificity towards a pathological site (bioavailability at the target site), the necessity of a large dose to achieve high local concentration, non-specific toxicity, inactivation of vectors due to undesired interactions with components of the *in vivo* milieu and other side effects due to high vector doses. Magnetofection  $^{\text{TM}}$  technology resolves the problems related to diffusion limited process and to restricted bioavailability at the target site.

#### PRINCIPLE

*In vivo* Magnetofection<sup>™</sup> has been designed for *in vivo* targeted transfection and infection. This original system combines magnetic nanoparticles and nucleic acid vectors that will be retained after injection at the magnetically targeted site. In this way, targeted delivery minimizes systemic

distribution and reduces toxicity. Furthermore, the magnetic forces will enhance the uptake of magnetic nanoparticles by the target tissue, and thus improve the efficiency of transfection/transduction. This allows reducing the required nucleic acid or virus doses and the process time of delivery which is crucial for improvement of *in vivo* nucleic acid delivery.

#### WHAT ARE THE APPLICATIONS?

Three optimized *in vivo* Magnetofection<sup>TM</sup> reagents have been designed according to defined applications:

#### Non viral applications

*In vivo* PolyMag - a cationic polymer-based magnetic nanoparticles formulation - and *in vivo* DogtorMag - a cationic lipid-based magnetic nanoparticles formulation have been developed for *in vivo* targeted transfection of various types of nucleic acids such as DNA, RNA and oligonucleotides.

*In vivo* **SilenceMag** is a rapid, simple and highly efficient method dedicated to transfect small RNA (siRNA, miRNA) into target cells/tissue *in vivo*.

#### **Viral applications**

*In vivo* ViroMag is an optimized nanoparticles formulation dedicated to viral vectors that allows high efficiency with low titer. It is particularly suitable for Lentiviral/Retroviral, Adenoviral and Adeno-Associated Viral (AAV) vectors.

3 Injection and magnet application

(2) Complexes Formation

PolyMag/ DogtorMag 1µL/µg DNA

#### Examples of applications

Target tissue	Route of injection	Site of injection	Kind of magnet	Magnet position
Tumor	Intravenous Intratumoral	Tail vein Tumor	All kinds	External (subcutaneous tumor, brain tumor, well localized tumor) Internal (interne organ tumor)
Endothelial cells	Intra-arterial	Vessel of interest Ear artery Femoral artery	All kinds	Internal (deep vessels) External (ear artery)
Heart	Intravenous Intra-arterial	Tail vein Carotid artery	Cylinder	Internal (in the chest) External (on the chest)
Liver	Intravenous Intra-arterial	Tail vein Carotid artery	Cylinder Square	External (on the right fiank) Internal (for focalized gene transfer)
Lung	Intravenous	Tail vein	Square	External
Intestine	lleum lumen	Intestine	Cylinder, Square	Internal
Brain	Intraventricular	Brain ventricle	Small Cylinder	External

Magnet can be positioned: 1) Externally for large organs or isolated organs (liver, brain, muscle, subcutaneous tumor), 2) Internally for deep organs or focalized gene transfer

#### HOW DO I USE IN VIVO MAGNETOFECTION™ REAGENTS?

Gene vectors/nanoparticles complexes can be easily administrated through various injection routes such as:

- **Systemic administration** (intravenous, intra-artery)
- •Local administration (intratumoral, intracerebroventricular, intraperitoneal, intramuscular, subcutaneous).

The only requirement for in vivo Magnetofection  $^{\text{TM}}$  is a small magnet specifically designed for this application. Several kinds of magnets are provided depending of your application (see page 9).

## In vivo PolyMag - In vivo DogtorMag

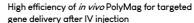
Two types of ready-to-use *in vivo* Magnetofection<sup>™</sup> reagents are offered:

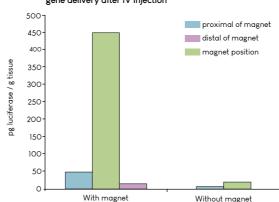
- In vivo PolyMag a cationic polymer-based magnetic nanoparticles formulation.
- *In vivo* **DogtorMag** a cationic lipid-based magnetic nanoparticles formulation. It associates *in vivo* Dogtor, a specific cationic lipid and *in vivo* CombiMag magnetic nanoparticles.
- ▶ To learn more about *in vivo* Magnetofection Technology see page 50

#### **APPLICATIONS**

- Suitable for various types of nucleic acids: plasmid DNA, siRNA, oligonucleotide, mRNA, shRNA, etc.
- Several routes of administration: Systemic & Local administration







After 42H, reporter gene expression was found primarily at the magnet position site and to a lesser extent proximal and distal of the magnet. As control, the same vector composition was injected in the contralateral vessel without application of a magnet. No significant reporter gene expression was found at the topographically analogous positions. From Plank et al., Expert Opin Biol Ther., 2003; 3:745-58

#### **PUBLICATIONS**

"Systemic delivery and activation of the TRAIL gene in lungs, with magnetic nanoparticles of chitosan controlled by an extrenal magnetic field"

#### In vivo DogtorMag

Ungureanu B.S. et al - Int.J.Nanomedicine. 2016

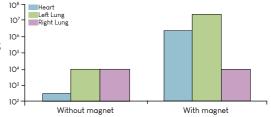
"Neuron-derived neurotrophic factor functions as a novel modulator that enhances endothelial cell function and revascularization processes"

#### In vivo PolyMag

siRNAs injection into left adductor muscle of mice. Ohashi K. *et al* - **J Biol Chem. 2014** 

▶ Browse our citation database online

#### Transfection of heart and lung in mouse



Luciferase expression after tail vein injection, both in presence or absence

#### MAIN FEATURES

#### • Increased transfection efficiency

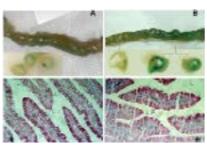
The magnetic forces enhance the uptake of magnetic nanoparticles by the target tissue and thus improve the efficiency of transfection

- Magnetically targeted transfection to specific area
- Reduction of the systemic dissemination of vectors during injection

Targeted delivery minimizes systemic distribution, decreases gene vectors inactivation & reduces toxicity

- Reduction of vector doses
- Work under non-permissive conditions
- Universal suitable for all nucleic acids Gene delivery/ODN delivery/Gene silencing
- Non-toxic, biodegradable & totally biocompatible

#### Transfection with in vivo PolyMag in rat intestine



Complexes of DNA and *in vivo* PolyMag nanoparticles were injected into the ilea of rats in absence (A) or under the influence of a magnetic field (B).

Magnetofection Technology - This reagent needs to be used with a specific magnet (p.9)

No. of injections Cat. No. Product 1 cylinder Magnet + 100 µL *In vivo* PolyMag IV-TK30210 In vivo PolyMag Trial Kit IV-TK30220 1 cylinder Magnet + 100  $\mu$ L *In vivo* Dogtor & *in vivo* CombiMag IV-PN30500 In vivo PolyMag 500 μL 5-50 IV-PN31000 In vivo PolyMag 1 mL 10-100 IV-DM30500 *In vivo* DogtorMag 500 μL IV-DM31000 In vivo DogtorMag 1 mL 10-100 IV-KC30210 In vivo PolyMag Starting Kit Magnets set + 500 µL In vivo PolyMag In vivo DogtorMag Starting Kit Magnets set + 500 uL In vivo Dogtor & In vivo CombiMag IV-KC30220

Magnet set contains 1 extra small cylinder (ø 2 mm), 1 small cylinder (ø 5 mm), 1 cylinder (ø 10 mm) and 1 square (18x18 mm) magnets

www.ozbiosciences.com

## Mag - In vivo infection

*In vivo* ViroMag has been designed to improve and target *in vivo* viral infection. This reagent is an optimized nanoparticles formulation dedicated to viral vectors. This original system combines magnetic nanoparticles and viral vectors that will be confined at the magnetically targeted site after injection.

▶ To learn more about *in vivo* Magnetofection Technology see page 54

#### **APPLICATIONS**

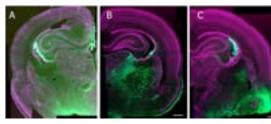
• *In vivo* transduction with all types of virus: Lentiviral/Retroviral, Adenoviral & AAV vectors

#### Several routes of administration:

Systemic & Local administration

RECOMMENDED APPLICATION
In vivo targeted infection/transduction

Infection of rat embryo Brain with Lentivirus



Brain sections at 8 days after lateral ventricular injection of 10° particles of GFP-lentivirus coupled with *in vivo* ViroMag into in utero rat embryos (E16) showed a diffuse GFP-expression (in green) due to a widespread infection of neurons (A). The association of GFP-lentivirus with ViroMag induced a targeted local area as shown by the GFP-expression in neurons lying under a magnet at the surface of the embryo skull (B). A more intense and restricted GFP-expression (C) was also observed when the magnet was positioned on the edge of the brain leading to an accumulation of viral particles and infected neurons in the focal area.

#### **PUBLICATIONS**

52

Brain infection with Lentivirus

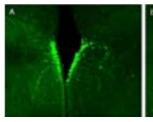
"Virus stamping for targeted single-cell infection in vitor and in vivo".

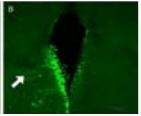
Schubert R.  $et\ al$  - Nature Biotechnol. 2018

"Magnetic nanoparticles for efficient cell transduction with Semliki Forest virus". Kurena B. et al - J Viral Methods. 2017

▶ Browse our citation database online

High efficiency of *in vivo* ViroMag for targeting viral vector after intracerebroventricular injection





Third ventricles of in utero rat embryos were injected with GFP-encoding viral particles alone or complexed to *in vivo* ViroMag. Without magnetic nanoparticles (A), The virus-transduced cells are located on both sides of the ventricle. Using *in vivo* ViroMag (B), transduction is enhanced and localized to one side due to a 30s magnet-application.

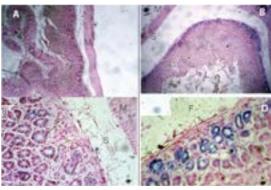
#### MAIN FEATURES

- Increased transduction/infection efficiency
   The magnetic forces enhance the uptake of magnetic nanoparticles by the target tissue and thus improve the efficiency of infection
- Magnetically targeted transfection to specific area
- Reduction of virus titer & systemic dissemination

Targeted delivery minimizes systemic distribution, allows reduction of the vector doses & reduces toxicity

- Work under non-permissive conditions
- Non-toxic, biodegradable & biocompatible

High infection efficiency in mouse stomach with in vivo ViroMag



In the absence of a magnetic field, gene delivery occurred in only a few transfected cells (A,C), while exposure to a magnet for 20min produces strong and widespread transgene expression (X-gal staining) in the crypts of the fundic glands 4 days after gene delivery (B,D)

Magnetofection Technology - This reagent needs to be used with a specific magnet (p.9)

 Cat. No.
 Product
 No. of inj

 IV-TK30230
 In vivo ViroMag Trial Kit
 1 Cylinde

 IV-VM30250
 In vivo ViroMag 250 µL
 10-25

 IV-VM30500
 In vivo ViroMag 500 µL
 20-50

 IV-KC30230
 In vivo ViroMag Starting Kit
 1 Magnet

No. of injections 1 Cylinder Magnet + 50 µL *In vivo* ViroMag 10-25

20-50 1 Magnets set + 250 µL *In vivo* ViroMag

Magnet set contains 1 extra small cylinder (ø 2 mm), 1 small cylinder (ø 5 mm), 1 cylinder (ø 10 mm) and 1 square (18x18 mm) magnets

## In vivo SilenceMag - In vivo gene silencing

In vivo SilenceMag<sup>™</sup> is a rapid, simple and highly efficient method dedicated to transfect small RNA (siRNA, miRNA) into target cells/tissue *in vivo*. It combines magnetic nanoparticles and small RNA that will be retained after injection at the magnetically targeted site. This targeted delivery method minimizes systemic distribution, increases gene targeted inactivation and reduces toxicity. Furthermore, the magnetic forces enhance the uptake of magnetic nanoparticles by the target tissue, and thus improve the efficiency of silencing. This allows decreasing the required process time of delivery to few minutes which is crucial for improvement of *in vivo* small RNA delivery. *In vivo* SilenceMag<sup>™</sup> is designed to meet *in vivo* grade quality.

▶ To learn more about *in vivo* Magnetofection Technology see page 54

#### **APPLICATIONS**

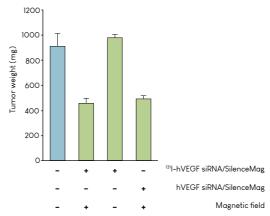
- In vivo gene silencing
- Several routes of administration: Systemic & Local administration

## RECOMMENDED APPLICATION In vivo gene silencing

#### MAIN FEATURES

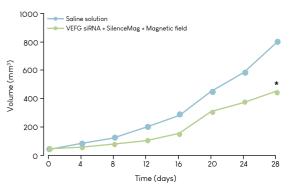
- Increased silencing efficiency
- Targeted process (magnetically-driven)
- Reduction of the systemic dissemination of siRNA/miRNA during injection
- Reduction of the siRNA/miRNA doses
- Work under non-permissive conditions
   Hypothermia, physiological flow conditions
- Penetration of the siRNA/miRNA into tissues
- Minimized toxicity

Tumor growth 28 days post treatment with hVEGF siRNA/SilenceMag



Results from Chen et al - BMC Cancer. 2014 14:114

#### Transfection of subcutaneous tumor in mouse



Subcutaneous tumors were generated by injection of hepatocarcinoma tumor cells into the right fiank of immunosuppressed mice. Tumor growth was then monitored daily after intravenous injection of VEGF/SilenceMag. From Chen et al – BMC Cancer.2014

#### **PUBLICATIONS**

"Kidney-specific Csf2 knockdown. In vivo gene silencing achieved by transfecting siRNA using in vivo SilenceMag".

Fujiu K. *et al* - **Nature Medicine. 2017** 

"In vivo SilenceMag enables to knock-down hVEGF expression into tumors bringing angiostatic & antitumoral effects".

Chen et al - BMC Cancer. 2014

Magnetofection Technology - This reagent needs to be used with a specific magnet (p.9)

No. of injections Contains 1 Cylinder Magnet + 100 µL reagent

5-50 10-100

Magnets set + 500 µL reagent

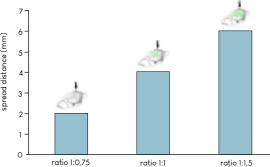
Magnet set contains 1 extra small cylinder (ø 2 mm), 1 small cylinder (ø 5 mm), 1 cylinder (ø 10 mm) and 1 square (18x18 mm) magnets

## BrainFectINTM - In vivo delivery into CNS

Major difficulties with gene delivery in the Central Nervous System (CNS) is the weakness of standard non-viral gene carriers and the limitations associated to the use of viral particles (time-consuming and requires additional safety precautions). Unlike these methods, BrainFectIN $^{\text{TM}}$  is an original non-viral formulation that allows safe, easy and efficient nucleic acids delivery into CNS of small animals. This transfection reagent allows transfection of neural cells in specific brain following stereotaxic injection, with low immunogenicity and rapid and long-term transgene expression. BrainFectIN $^{\text{TM}}$  has been designed to meet *in vivo* grade quality.

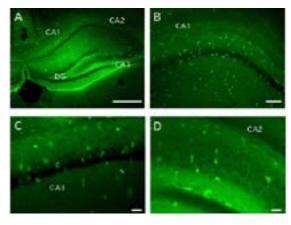
## RECOMMENDED APPLICATION In vivo nucleic acids delivery-brain specific

Quantitative analysis of BrainFectIN  $^{\rm TM}$  /DNA spread into the rat hippocampus



After injection, BrainFectIN  $^{\! \mathrm{TM}}/p$  GFP complexes can spread into the whole hippocampus structure from rostro-caudal to lateral direction.

## Stereotaxic injection of BrainFectIN $^{\text{TM}}$ /DNA complexes in hippocampus



GFP-expression in hippocampus of rat 48H after BrainFectIN/pGFP injection (ratio 1:1.5). Scale bar = 100µm. The mix was injected through a nanofil needle implanted into hippocampus (stereotaxic injection). GFP+ cells are located in Dentate Gyrus (A) as well as hippocampal areas CA1 (A,B,C), CA2 (A) and CA3 (A,D). Negative control has been done with a stereotaxic injection of DNA alone in the same conditions. It shows a few cells transfected.

#### TESTIMONIAL

"BrainFectIN™ was successfully tested in my lab for in vivo stereotaxic purpose and is now extensively used to modify neuronal cells. This reagent provides a new approach leading to an efficient transfection rate and a large diffusion scale from ipsi to contralateral hemisphere by adjusting the injected volume. Our plasmid DNA- which can be detected for weeks after injection- is expressed shortly after transfection when compared to a viral approach."

Christophe P., PhD - University of Aix-Marseille - France

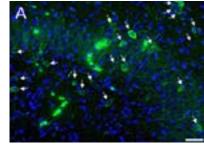
"A new polymer-based approach for in vivo transfection in postnatal brain".

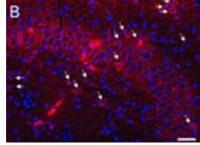
Di Scala C. et al- J.Neuro Methods. 2018-Submitted

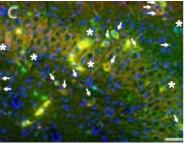
## MAIN FEATURES

- Targeted process (stereotaxic injection)
- Good transfection efficiency
- Reduction of the injection volume
- Reduction of the DNA doses
- Minimized toxicity
- Low immunogenicity
- Rapid and long-term transgene expression

#### Double immunofluorescence staining performed in CA3 area







Transfected cells are GFP+ (A, arrows), and interneurons are labelled with GAD 65/67 (B, arrows), nuclei are counterstained with Hoecsht (A,B,C). Merge shows that we are able to transfect GABAergic interneurons (C, arrows). By exclusion, every other cell GFP+ is either pyramidal cell or hippocampal granule cell (C, asterix). It shows that BrainFectIN allows to transfect at least 3 different neural cell types after intra-hippocampal injection. Scale Bar = 50µm

Cat. No. IV-BF30100 IV-BF30250 IV-BF30500

In vivo BrainFectIN 100 μL In vivo BrainFectIN 250 μL In vivo BrainFectIN 500 μL No. of injections 20–30 40–60 80–120

## VIRAL APPLICATIONS

#### Infection & Transduction Enhancers

Magnetofection™

ViroMa

ViroMag R/I

ViroMag Ster

AdenoMa

Lentivirus / Retrovirus

LentiBlast Premium

LentiBlast Premium Superior Grad

Adenovirus / AAV

AAVBlast

#### Cell sorting & Transduction

ViroMICST™

ViroMICST™Stem

#### Capture & Concentration

Mag4C - AD

Vlag4C – L\

ViroPeg Lentivirus Concentrator

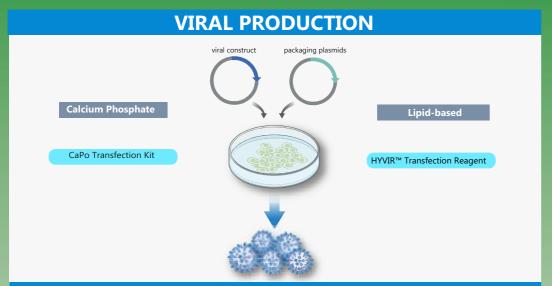
#### Virus Production

Calcium Phosphate Transfection Ki

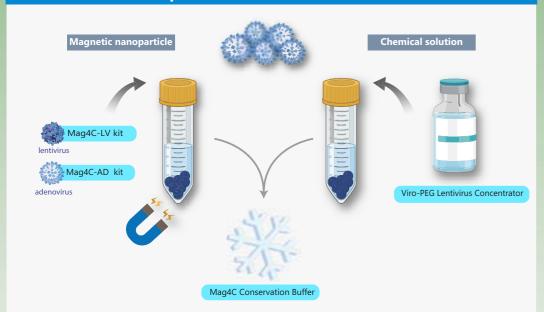
#### Preservation & Storage

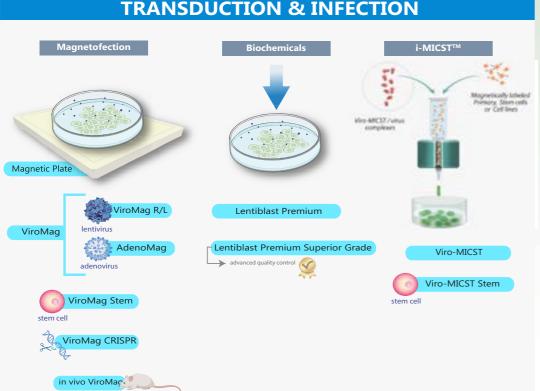
MAG4C Conservation Buffe

## VIRAL APPLICATION



#### VIRUS CAPTURE, CONCENTRATION & PRESERVATION







ViroMag is a versatile reagent offering a solution for many viral applications. ViroMag and virus to be transduced are mixed in a one-step procedure; no molecular biology processes or biochemical modifications are required. This reagent demonstrates an exceptionally high efficiency to promote, control and assist viral transductions. ViroMag is applicable to all viral vectors and presents unique properties due to a specific and optimized magnetic nanoparticles formulation.

▶ To learn more about Magnetofection Technology see page 9

#### **APPLICATIONS**

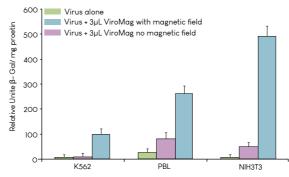
• Suitable for all viral vectors: Adenovirus, α-virus, Baculovirus, Herpes virus, Lentivirus, Retrovirus, Rhabdovirus, Paramyxovirus, Polyomavirus...

Mammalian cells: adherent and suspension

· primary cells, hard-to-transfect cells and cell lines

## RECOMMENDED APPLICATION To increase viral transduction efficiency without Polybrene

#### ViroMag effect on Adenovirus transduction



Cells were infected with an adenovirus alone (Ad-LacZ) or with complexes of adenovirus and 3 µL of ViroMag. Cells were submitted or not to magnetic field and β-Galactosidase expression was determined 24H after infection

#### **PUBLICATIONS**

"Infection with ViroMag allowed to use 10 times less viruses with better infectivity and viability."

#### Herpes virus

Sloutskin A et al - J Virol Methods. 2014

"Synchronous infection of SIV and HIV in vitro for virology, immunology and vaccine-related studies."

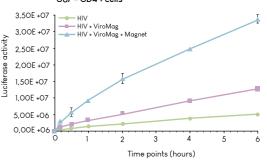
#### T Lymphocyte CD4+

Sacha J.B. et al - Nature Protoc. 2010

"Efficient lentiviral transduction of Hematopoietic Stem Cells with Magnetofection" Hosokawa K. et al - Nature Com. 2017

▶ Browse our citation database online

#### ViroMag accelerates HIV infection process on U87 - CD4 + cells



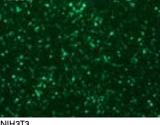
#### MAIN FEATURES

- Increases viral transduction efficiency Up to 500-fold gene expression enhancement compared to standard infection
- Improves viral infectious capacity Promotes infection even with very low viral doses
- Concentrates viral dose, promotes and accelerates the infection process Increases viral dose concentration on cell surface and uptake by 70-100 fold
- Extends the host tropisms to non-permissive
- -Association of certain viruses with ViroMag is sufficient to infect cells lacking viral receptor -Enhances ability to transduce *in vitro* target cells without modifying viruses
- Allows synchronization of transduction
- -Synchronizes viral cell adsorption (uptake)
- -Accurately monitor the kinetics of viral replication

#### • Can provide a magnetic targeting

High transduction can be achieved under magnetic influence and confined to specific area by the magnet shape and position

- For *in vivo* applications please refer to *in vivo* ViroMag page 56
- ▶ ViroMag Stem is also available for stem cell application (VMST100)



Magnetofection Technology - This reagent needs to be used with a magnetic plate (p.9)

	_ 10 1 1 1
Cat. No.	Product
VM40100	ViroMag 100 μL
VM40200	ViroMag 200 µL
VM41000	ViroMag 1 mL
KC30500	ViroMag Starting Kit
KC30600	ViroMag Triple Starting Kit

\*Based on MOI of 1 for 104 cells/well

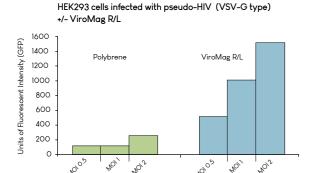
No. of assays\* 30-500 transductions in 96-well plate 60-1000 transductions in 96-well plate 300-5000 transductions in 96-well plate 1 magnetic plate + 200 uL ViroMag 1 magnetic plate + 100µL ViroMag+AdenoMag+ViroMag RL

56

## <sup>™</sup>ViroMag R/L - Retrovi<u>rus & Lentivirus infection enhance</u>r

ViroMag R/L transduction reagent is a magnetic nanoparticles formulation optimized for Retroviruses and Lentiviruses. Based on the Magnetofection™ technology, this reagent allows concentrating the complete applied dose of Retro/Lentiviral particles onto cells within minutes, inducing a significant improvement of virus infectivity with extremely low vector doses.

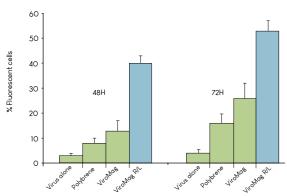
▶ To learn more about Magnetofection Technology see page 9



#### MAIN FEATURES

- Increases viral transduction efficiency Increases percentage of transduced cells
- Improves viral infectious capacity Significantly enhances virus infectivity even with very low viral doses
- Concentrates viral dose Increases retroviral titer from culture supernatant by 1000 to 4000 fold
- Promotes and accelerates the infection process
- Allows synchronization of transduction
- Straightforward and non-toxic No molecular biology or biochemical processes
- Can provide a magnetic targeting

#### ViroMag R/L is highly efficient for lentiviral infection



NIH-3T3 cells were infected with a lentivirus coding for GFP alone or with Polybrene, ViroMag and ViroMag R/L. Percentage of infected cells was determined 48 and 72H after infection by FACS analysis.

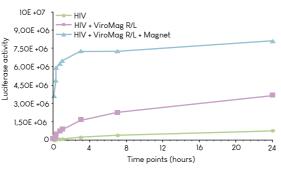
#### **APPLICATIONS**

- Perfect for cell transduction with all retroviral and lentiviral vectors: especially VSG-G pseudo viruses
- Suitable for mammalian cells: cell lines, primary cells, hard-to-transfect, suspension cells

## Successfully tested and published!

RECOMMENDED APPLICATION Enhancing and synchronizing retro and lentiviruses transductions

> ViroMag R/L accelerates pseudo VSV-G HIV infection on U87-CD4<sup>+</sup> cells



#### **PUBLICATIONS**

"High transduction efficiency on mammary epithelial organoids in suspension." Shamir E.R. et al - J Cell Biol. 2014

"Efficient transduction of crypt cells and mouse organoids with ViroMag R/L magnetic nanoparticles." Lingling Xian et al - Nature Com. 2017

"Bone marrow transduced by Magnetofection using ViroMag R/L."

Sugimura R. et al - Cell. 2012

▶ Browse our citation database online

### Magnetofection Technology - This reagent needs to be used with a magnetic plate (p.9)

Cat. No.	
RL40100 RL40200	
RL41000 KC30700	

ViroMag R/L 100  $\mu$ L ViroMag R/L 200 μL ViroMag R/L1 mL ViroMag R/L Starting Kit

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No. of assays 30-500 transductions in 96-well plate 60-1000 transductions in 96-well plate 300-5000 transductions in 96-well plate Contains 1 magnetic plate + 200 µL ViroMag R/L

## AdenoMag - Adenovirus & AAV infection enhancer

AdenoMag is a magnetic nanoparticles based reagent dedicated to enhance Adenovirus and Adeno Associated Virus (AAV) infection. It allows to concentrate rapidly all viral particles onto cells. AdenoMag permits to improve significantly virus infectivity with extremely low vector doses. Due to its specific properties, AdenoMag is ideal to infect non permissive cells. No molecular biology processes or biochemical modifications are required.

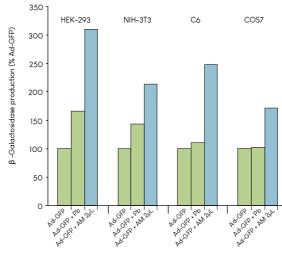
▶ To learn more about Magnetofection Technology see page 9

#### **APPLICATIONS**

- · Ideal for boosting your cell transduction with all adenoviral and AAV vectors
- Suitable for all mammalian cells: cells lines, primary, hard-to-transfect & non-permissive cells

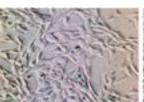
#### RECOMMENDED APPLICATION Adenovirus and Adeno-Associated-Virus transductions in vitro

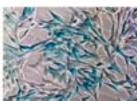
#### Adenomag enhances transgene expression



with Ad-LacZ, Ad-LacZ with Polybrene (Ad-LacZ + Pb) or Ad-LacZ with 2 µL of AdenoMag (Ad-LacZ + AM).

#### Comparison of NIH-3T3 infection with or without AdenoMag





#### MAIN FEATURES

#### Increases transduction efficiency

The combination of magnetic nano-particles with adenovirus showed up to 500-fold enhancement of gene expression compared with standard infection

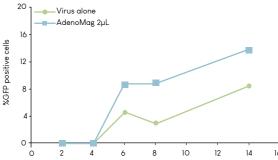
#### Concentrates viral dose

- -Promotes and accelerates the infection process -Improves viral infectious capacity
- Significant enhancement of adenovirus infectivity can be achieved with the use of magnetic nanoparticles

#### Extends the host tropisms of viral vectors to non-permissive cells

The association of viral vectors with magnetic nanoparticles is sufficient to permit infection of non-permissive cells

Provides a magnetic targeting



NIH-3T3 cells infection kinetics (MOI = 1) +/- AdenoMag

## **PUBLICATIONS**

"Magnetic nanoparticles enhance adenovirus transduction in vitro and in vivo."

Sapet C. et al - Pharm Res. 2012

"3T3-L1, C2C12: High transduction efficiency with AdenoMaa ."

Sasaki Y. et al - Plos One. 2015

▶ Browse our citation database online

#### Magnetofection Technology - This reagent needs to be used with a magnetic plate (p.9)

No. of assays\*

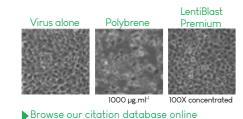
Cat. No.	Product	
AM70100	AdenoMag 100 µL	
AM70200	AdenoMag 200 µL	
AM71000	AdenoMag 1 mL	
KC30900	AdenoMag Starting K	(i
*Based on MOI of 1 for 10 <sup>4</sup> cells/well		

500-1000 transductions in 96-well plate 1000-2000 transductions in 96-well plate 5000-10000 transductions in 96-well plate Contains 1 magnetic plate + 200 uL AdenoMag

LentiBlast Premium is ideal to enhance lentiviral infection and transduction in any type of cells, adherent or in suspension, primary or cell lines. Its patent-protected chemical composition allows simultaneously neutralizing electrostatic repulsions between membrane and viral particles and enhancing viral fusion with cell membrane. Due to a favorable "membrane permeable effect" limiting the transmembrane potential changes, LentiBlast Premium is totally compatible with cell viability. It overcomes obstacles that prevent successful transduction (cell density, passage number, lentivirus purity, MOI ...).

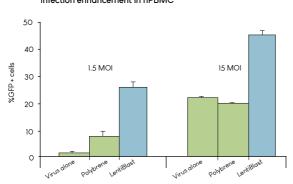
#### MAIN FEATURES

- Enhances infection & transduction efficiency
- Compatible with cell lines and primary cells
- Allows using reduced amounts of virus
- Non-toxic even at high concentration



#### RECOMMENDED APPLICATION Enhancing lentiviral transduction in any type of cells

Infection enhancement in hPBMC



#### **TESTIMONIALS**

"We use LentiBlast to help achieve high transduction efficiency of human primary T cells. It has lower toxicity than Polybrene which is traditionally use to enhance transduction & efficiency was doubled compared to Polybrene." Nina F - Albert Einstein College of Medicine

"Discover how using LentiBlast to boost lentivirus infection efficiency in human fibrosarcoma." Arnoult et al - Nature. 2017

> LBPX500 LBPX1500

Product LentiBlast Premiem 500 µL LentiBlast Premium 1500 µL No. transduction in a 24-well plate Up to 100 Up to 300



## LentiBlast Premium Superior Grade

LentiBlast Premium Superior Grade is the ideal reagent to enhance lentiviral infection and transduction in CD34+ hematopoietic stem cells, T lymphocytes as well as any other cells.

It is identical in synthesis and formulation to LentiBlast Premium Transduction Enhancer with advanced quality controls. It is designed for use in preclinical and early phase clinical trials and can be used as an ancillary material for cell & gene therapy.

#### RECOMMENDED APPLICATION

Ideal to Increase R&D, Preclinical and Clinical Transduction Protocols for Ex-vivo Gene Therapies & CAR-T Cell Therapies.

Cat. No. LBPSG50

LentiBlast Premium Superior Grade 5 ml LentiBlast Premium Superior Grade 1500 µL

Up to 1000 Up to 300

No. transduction in a 24-well plate

**AAVBlast** 

AAVBlast is a chemical AAV transduction enhancer, very effective to promote viral-mediated genetic modification. AAVBlast improves transduction in a wide range of cell types from classic cell lines to primary cells or mesenchymal stem cells.

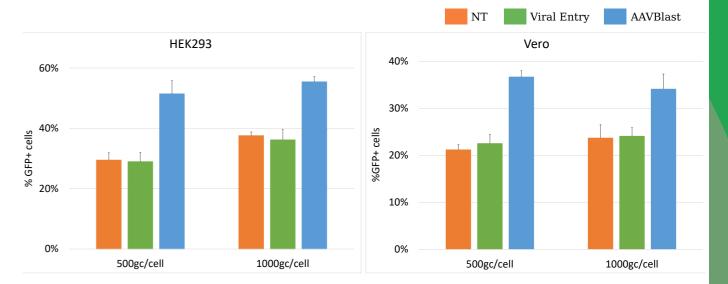
Its patented formulation and unique thermoresponsive gelling properties, ensure the protection of viral particles and allow an increase in transduction efficiency.

Non-toxic, the new AAVBlast AAV transduction enhancer is also compatible with in vivo experiments.

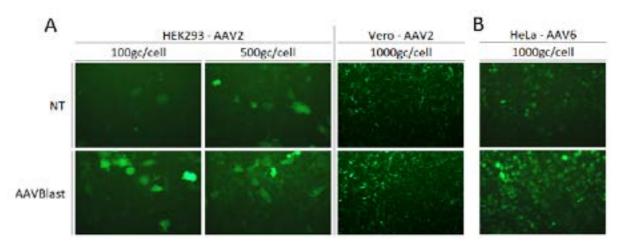
#### MAIN FEATURES

- Increase AAV infection efficiency for hard-totransduce cells: The right solution for Stem cells, Primary T cells
- Save effort & money: Achieve same transduction results with a lower viral titer
- Maximize Cell Viability: Non-toxic

RECOMMENDED APPLICATION Enhancing AAV transduction in any type of cells



HEK293 (A) and Vero cells (B) were transduced with AAV2 at two MOI in presence or not of commercial viral enhancer (VE) or AAVBlast according to their respective protocol. % of GFP+ cells was determined 72h after transduction by flow cytometry.



HEK293 & Vero cell (A) and HeLa cells (B) were transduced resepctively with AAV2 and AAV6 in presence or not of AAVBlast according to the protocol. 72H after transduction, GFP expression was monitored under fluorescent microscopy

Cat. No. Product No. of transductions in a 24-well plate AVVB250 AAVBlast 250 µL 400

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i-MICST™ technology (integrated Magnetic Immuno-Cell Sorting and Transfection/Transduction) is a new platform that allows to genetically modify cells directly on magnetic cell purification columns. This technology combines cell isolation and genetic modification in one simple, efficient and reliable integrated system. Designed for i-MICST™ technology, the Viro-MICST™ reagent allows efficient and specific transduction of target cells directly on magnetic cell-purification columns. Ideal for sensitive cell types such as primary and Stem Cells, Viro-MICST™ leads to an increase in the transduction efficiency with low-titer virus preparations compared to regular transduction methods.

- ▶ To learn more about i-MICST™ Technology see page 20
- ▶ ViroMisct Stem is also available for stem cell applications (VMX100)

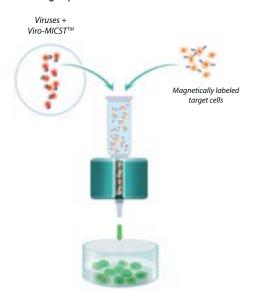
#### **APPLICATIONS**

- Suitable for all viruses: including AAV, Adenovirus, Lentivirus and retrovirus
- Ideal for mammalian cells: adherent and suspension cells, primary and hard-to-transfect cells, cells lines, sensitive cells

#### RECOMMENDED APPLICATION

Transduction/Infection of cells during magnetic cell purification

Integrated Magnetic Immuno-Cell Sorting and Transduction in one single system



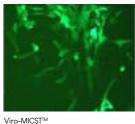
#### **PUBLICATION**

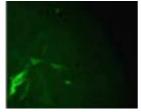
"Magselectofection: an integrated method of nanomagnetic separation and genetic modification of target cells."

Sanchez-Antequera Y et al - Blood. 2011

▶ Browse our citation database online

#### hUC-MSC adenoviral transduction improved by ViroMICST



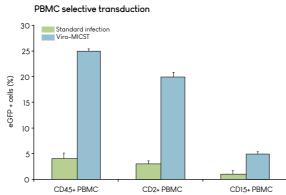


Standard infection

#### MAIN FEATURES

- Isolation and transduction of cells in one reliable integrated system
- -Reduce cell manipulation steps, minimize cell stress and save time
- -Ideal for sensitive cell types such as primary and
- High and increased transduction efficiency
- -Benefit from high transduction efficiency with low multiplicity of Infection (MOI) during magnetic cell
- -Save vector material
- Acceleration of the transduction process and synchronization of adsorption
- Cell phenotype maintained

Cells maintain their differentiation potential after using Viro-MICST procedure



Human PBMC were labeled with either CD45, CD2 or CD15 microbeads. Each condition was then loaded into (1) one unmodified MACS® LS column, and selected cells were then infected using standard lentiviral (standard infection) or (2) one unmodified MACS® LS column followed by a MACS® LS column modified with Viro-MICST/LV. eGFP complexes. Infection efficiency was measured

Cat. No. Product VMX250 Viro-MICST 250 µL Viro-MICST 500 µL VMX500 Viro-MICST 1000 µL No. of transductions per small column\* 25-50 50-100 100-200

\*Based on MOI of 1 for 106 labeled-cells/column

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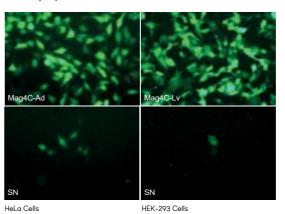
## Mag4C - Magnetic Virus Concentration

Mag4C Kit is specifically designed and developed for capturing, concentrating and storing viruses. This kit is composed of three reagents allowing Magnetic Capture/Concentration, Elution and Conservation of viruses. Mag4C magnetic nanoparticles capture viruses in culture media with 80-99% efficiency. Once captured onto magnetic beads, viruses can be:

- · Concentrated and stored with the conservation buffer or directly used for downstream assays
- · Concentrated, eluted from the magnetic beads with the elution buffer and stored with the conservation buffer or used for various assays

#### ▶ To learn more about Mag4C Technology see page 18

#### Efficiently captures viruses

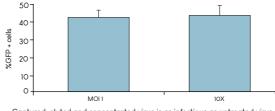


Mag4C beads efficiently captured virus since supernatants (SN) are nearly no more infectious (absence of virus) whereas viral particules bound to the Mag4C beads are highly infectious.

#### MAIN FEATURES

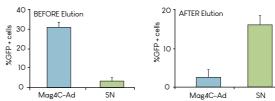
- Concentration viruses by magnetic capture in 30-45 minutes
- · Obtain high yield of viral capture and recovery
- Reduce handling steps
- Avoid ultracentrifugation precipitation &
- Mag4C beads improve transduction efficiency (Magnetofection advantages see page 7)

#### %HeLa infection after Concentration



Captured, eluted and concentrated virus is as infectious as untreated virus

#### HEK-293 cells infection



Elution is straightforward, easy and very efficient. Mag4C beads were no more

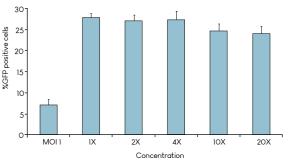
#### **APPLICATIONS**

- Suitable for all conditions & viruses:
- 2 differents products available:
- Mag4C-Ad for adenoviruses/AAV
- Mag4C-Lv for lenti- & retro-viruses

#### RECOMMENDED APPLICATION Fast capture, concentration

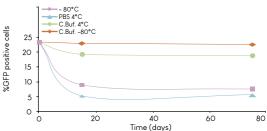
& conservation of viruses

#### % of COS7 infected after capture and concentration



After capture, virus complexed to Mag4C beads can be concentrated without

#### % COS7 infection after Conservation in Conservation Buffer or PBS



Viral particles stored in conservation buffer maintain high infectivity ove

#### **CONSERVATION BUFFER**

Mag4C Conservation Buffers (Lv&Ad) have been expressly designed to improve the stability of viruses upon storage conditions and are fully compatible with the magnetic nanoparticles.

LVB1000: Mag4C-Lv Conservation Buffer 1mL ADB1000: Mag4C-Ad Conservation Buffer 1ml

Cat. No.	Product	Description	No. of captures
ATK11200	Mag4C-Ad trial kit	Magnetic beads 0.2 ml + Buffers (Elution 5mL + Conservation 0.2 mL)	Up to 20
LTK11200	Mag4C-Lv trial kit	Magnetic beads 0.2 ml + Buffers (Elution 5mL + Conservation 0.2 mL)	Up to 20
AKC11000	Mag4C-Ad kit 1mL	Magnetic beads 1 mL + Buffers (Elution 5mL + Conservation 1 mL)	Up to 100
LKC11000	Mag4C-Lv kit 1 mL	Magnetic beads 1 ml + Buffers (Elution 5mL + Conservation 1 mL)	Up to 100
AKC11010	Mag4C-Ad kit 10 mL	Magnetic beads 10 ml + Buffers (Elution 50mL + Conservation 10 mL)	Up to 100
LKC11010	Mag4C-Lv kit 10 mL	Magnetic beads 10 ml + Buffers (Elution 50mL + Conservation 10 mL)	Up to 100
AKC11050	Mag4C-Ad kit 50 mL	Magnetic beads 50 ml + Buffers (Elution 250mL + Conservation 50 mL)	Up to 100
LKC11050	Mag4C-Lv kit 50 mL	Magnetic beads 50 ml + Buffers (Elution 250mL + Conservation 50 mL)	Up to 100

A Multipurpose Magnetic Separation Rack for 50, 15 or 1.5 mL tubes is also proposed (p.19)

## ViroPeg Lentivirus Concentrator

The Viro-PEG Lentivirus Concentrator is a ready-to-use solution optimized for the capture and concentration of lentiviral particles, providing an easy and straightforward method to efficiently concentrate lentiviral particles without using ultracentrifugation.

#### **APPLICATIONS**

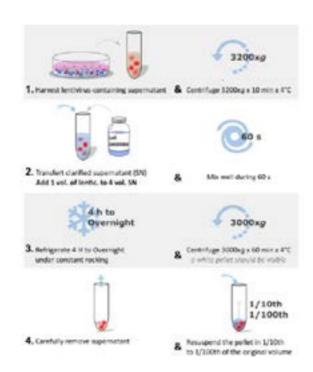
#### **Ideal method for lentivirus concentration:**

Virus can be concentrated over 100 fold with an efficiency ranging from 70 to 95%

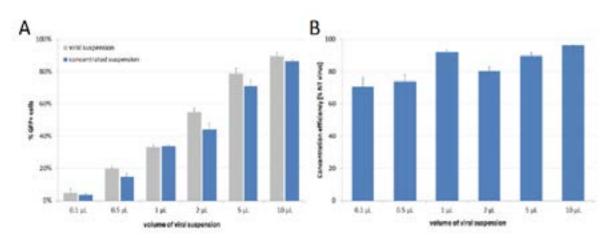
### RECOMMENDED APPLICATION

#### MAIN FEATURES

- Ideal for virus concentration
- Increase lentiviral titers by up to 100 fold
- Simple & convenient protocol adaptable from 4 hours to overnight incubation
- Allow rescuing low-titer viral particles
   No ultracentrifugation is required
- Ready-to-use & non-toxic to target cells



Lentiviral infection efficiency after concentration using ViroPeg varies between 70 % and 95 % when compared to non-treated lentivirus.



#### Jurkat T cells infection after lentiviral concentration and concentration efficiency

Lentiviral suspension was concentrated 10 times using ViroPeg (1 vol lenticoncentrator for 4 volumes of viral suspension). Concentrated virus was diluted 10 times to bring the volume back to the initial one and the infection capacity was compared to initial viral suspension according to ranging volumes (A). Concentration efficiency compared to non-treated (NT) virus was calculated (B).

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Cat. No. Product
LVG100 100 mL of Viro-PEG Lentivirus Concentrator
LVG500 500 mL pf Viro-PEG Lentivirus Concentrator

## Calcium Phosphate Transfection Kit - Virus Production

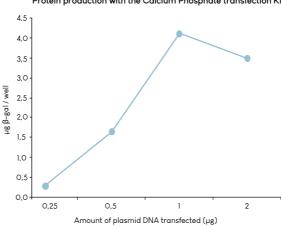
Calcium Phosphate transfection Kit is perfect to transfect HEK-293 cells. This transfection method, first described by Graham and Van Der Ebb in 1973, has been optimized in order to reach higher transfection efficiency. The CaPO transfection Kit is simple and easy to use. It allows reaching between 95 and 100% of HEK 293 transfected cells and a very high titer for virus production.

#### **APPLICATIONS**

 Ideal method for HEK 293 cells transfection: Calcium Phosphate transfection Kit is optimized for the transfection of HEK-293 cells with plasmid DNA. It is also appropriate for a variety of immortalized cell lines such as CHO and COS cells

# RECOMMENDED APPLICATION Transfection of HEK-293 cells for production of viral vectors and proteins

Protein production with the Calcium Phosphate transfection Kit



HEK-293 cells were prepared and transfected in 24-well plates with several amount of a pLACZ plasmid encoding β-Galactosidase. The amount of β-galactosidase produced per well was determined by ONPG assay (p.69#G010001).

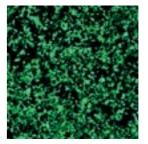
#### FOCUS ON

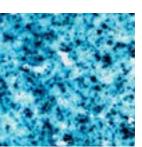
#### Principal advantages:

- •Compaction of DNA in nanoparticles efficiently internalized by cells
- Protection of nucleic acids against nucleases de-
- •Modified & optimized to reach higher transfection levels

For virus production, see also HYVIR Transfection Reagent, page 48

HEK-293 cells transfected with the Calcium Phosphate transfection Kit





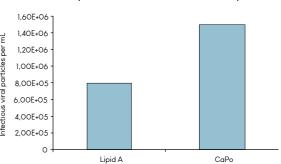
GFP

β-galactosidase

#### MAIN FEATURES

- Ideal for virus production
- High HEK-293 cells transfection efficiency
- Suitable for producing recombinant proteins
- Serum compatible
- Simple, rapid and ready-to-use:
  - 1.Plate the cells in DMEM and incubate overnight
  - $\begin{tabular}{ll} 2. Change tissue culture medium 1-2H before \\ transfection \end{tabular}$
  - 3.Prepare the DNA solution in 1X HBS
  - $4.\mbox{Add}$  the Calcium Chloride solution, mix and incubate 30 min
  - 5.Add the complexes drop wise to your cells

#### Virus production with CaPO Kit versus competitor



A Lentiviral expression plasmid, a packaging plasmid, and a pseudotyping plasmid were mixed together (20 μg total DNA amount) and transfected in a 100 mm dish with the CaPO kit. As a control the same amount of the three plasmids were transfected with a competitor's reagent as indicated by the manufacturer's instruction manual. Viral particles were collected after 48H & viral titers were determined using HeLa-CD4 β-galactosidase cells (MAGI assay)

Cat. No.

Product CaPO Transfection Kit

No. of assays 100 in 100mm culture dishes with 1µg of DNA

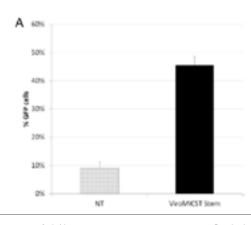
Kit content: 1X Hepes Buffered Saline 4x15 mL+2.5 M CaCl2 3.5 mL

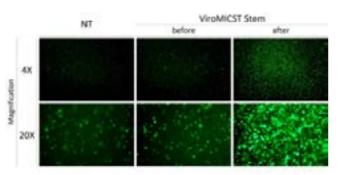
Viro-MICST™ Stem Transduction Enhancer is a new specific magnetic nanoparticles formulation issued from our Magnetofection™ technology. Its properties were specifically designed for Stem Cell lentiviral transduction in order to achieve high infection rate directly on magnetic cells sorting devices (column or magnet). As a stabilized magnetic nanoparticles formulation, ViroMICST Stem offers a simple and reproducible method for increasing lentiviral infection and transduction of difficult cell types such as CD34+ hematopoietic stem cells, both cell lines and primary cells, during cell sorting.

▶ To learn more about i-MICST<sup>™</sup> Technology see page 20

#### **APPLICATIONS**

- Suitable for Lentivirus and Retrovirus.
- Ideal for CD34+ Hematopoietic Stem Cell transduction





#### MAIN FEATURES

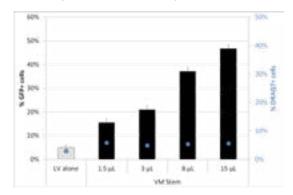
- Increased Lentiviral Infection
- Reduce cell manipulation steps and save time and material
- Designed for stem cells

Cat. No. VMXS100 VMX300 Product Viro-MICST Stem Viro-MICST Stem No. of transductions per small column\* 100 300

\*Based on MOI of 1 for 10° labeled-cells/column

## ViroMag Stem - Transduction enhancer for stem cells

ViroMag Stem, a Magnetofection-based Lentiviral Transduction Enhancer, enables improved viral driven genetic modification in a wide range of stem cells in different applications, such as ex vivo gene therapy and cell therapy. As a stabilized magnetic nanoparticles formulation, it offers a simple and reproducible method for increasing lentiviral infection and transduction of difficult cell types such as CD34+ hematopoietic stem cells, both cell lines and primary cells, in cell culture plate using the Magnetofection technology. This method combines maximum cell viability and high transduction efficiency while cell phenotype and differentiation potential are not affected.



 $\label{thm:comparison} \mbox{ViroMag Stem does not impair CD34+ stem cells survival after lentiviral mediated transduction.}$ 

RECOMMENDED APPLICATION
Ex vivo gene therapy and cell therapy

#### MAIN FEATURES

- Increase lentiviral transduction efficiency
- Concentrate viral dose, promote and accelerate the transduction process
- Efficient for hard-to-infect and nonpermissive cells
- · Synchronise cells adsorption/infection

No. of transductions per small column\* Cat. No. Product VMXS100 Viro-MICST Stem 100 VMX300 Viro-MICST Stem 300 VMST100 ViroMag Stem 100 VMST300 ViroMag Stem 300 KC31000 ViroMag Stem Starting Kit 100 \*Based on MOI of 1 for 10<sup>6</sup> labeled-cells/column

## LIPID NANOPARTICULES

Custom LNP

Ready-to-use LNP

LNP-mRNA

Empty LNF

Fluorescent LNF

LNP DIY (Do It Yourself

www.ozbiosciences.com

## **LNP Design**



#### Lipids



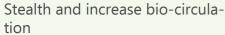
**Complexing lipid (Ionizable)** Nucleic acid complexation Membrane fusion & endosomal escape **OZ Biosciences** proprietary lipids



**Helper lipid** Stability and structure



#### **PEG-lipid**





#### Cholesterol

Rigidity and integrity Help the endosomal release

## Cargo



**Nucleic acid** DNA, ODN,

RNA (siRNA, mRNA, saRNA, shRNA, miRNA...), ...



#### **Small molecules**

peptide protein ...

## **LNP Manufacturing**



#### **Solutions preparation**

Cargo in buffer

**Lipids in ethanol** 





## **Microfluidic Mixing**

N/P ratio





**Loaded LNP** 

**Size Checking** 

## **Downstream Process**



#### **Purification and Formulation**

#### **Dialysis and TFF**



Removal of alcohol solvent and free molecules, addition of cryoprotectant, formulation at desired concentration

#### **Sterilization**

0.2 µm filter







## Stabilized LNP in neutral pH

\* LNP can be optionally **functionalized** with targeting ligands such as peptides, antibodies...

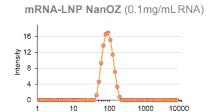
## **Quality Control**



#### **Caracterization studies**

#### **Dynamic Light Scattering**

Hydrodynamic size, polydispersity index (PdI) and zeta potential

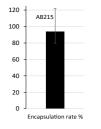


#### **Encapsulation Efficiency**

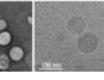
DNA- or RNAquantification kit assay

## On request

CryoTEM, negative stain EM, HPLC, LC-MS







## **Sterility studies**

Thioglycolate and Caso-bouillon

## **Stability studies**

RT ; 4 °C ; -20°C ; -80°C

2 to 3 weeks



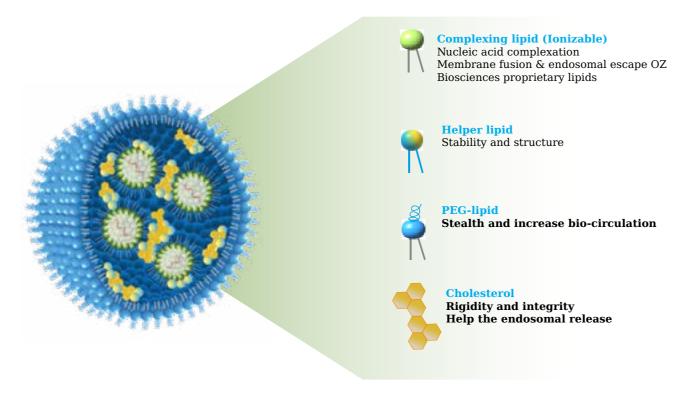
Watch our video online!



Lipid Nanoparticles (LNPs) represent the most effective and safe delivery systems for the translational success of nucleic acid drugs. NanOZ LNP-RNA is designed not only to protect RNA from degradation, but also to facilitate intracellular uptake and thus, potentiate its efficacy. Our delivery systems are produced through microfluidic technology resulting in monodisperse NanOZ LNP-RNA with narrow size distribution and high encapsulation efficiency (>85%).

#### **MAIN FEATURES**

- Liposome-like structures, engineered for encapsulating a broad variety of nucleic acids (RNA, mRNA, siRNA, gRNA, cRNA and DNA) and APIs.
- LNP consists in inner core surrounded by a lipidic shell based on a combination of four families of chemicals, each having distinct functions.



#### **APPLICATIONS**

• For In Vitro: variety of cells (RAW 264.7, THP-1, PANC-1, HeLa)

For T cells -> please contact us for optimized solutions

• For In Vivo: suitable for I.P, S.C, I.M,

For I.V -> please contact us for optimized solutions



NanOZ LNP-mRNA(GFP): mRNA encoding GFP protein formulated in Lipid Nanoparticle.



NanOZ LNP-mRNA(Luc): mRNA encoding Firefly Luciferase protein formulated in Lipid Nanoparticle.



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NanOZ LNP-mRNA(OVA): mRNA encoding Ovalbumin protein formulated in Lipid Nanoparticle.



NanOZ empty-LNP: empty LNP designed to be used as a control vehicle in various experiments involving studies of LNP-nucleic acid therapeutics

Cat. No. LNP10500MRNA11 LNP10500MRNA12 LNP10500MRNA41 LNP10500

Product 0.5mL (2\*250µL) of LNP-mRNA, (100µg/mL of 5moU GFP mRNA) 0.5mL (2\*250µL) of LNP-mRNA, (100µg/mL of 5moU Luc mRNA) 0.5mL (2\*250µL) of LNP-mRNA, (100µg/mL of 5moU OVA mRNA) 0.5mL (2\*250µL) of empty LNP

Available in 0.5mL, 1mL and 5 mL

#### Fluorescent LNP



**NanOZ Fluo LNP-DiR**: is designed for the effective monitoring and imaging of intracellular trafficking and biodistribution of LNPs in vitro and in vivo respectively. It contains a lipophilic carbocyanine dye DiR having an excitation max. at 748 nm, and emission max. at 780 nm.

NanOZ Fluo LNP-DiO: is designed for the effective monitoring and imaging of intracellular trafficking and biodistribution of LNPs in vitro and in vivo respectively. It contains a lipophilic carbocyanine dye DiO having an excitation max. at 487 nm, and emission max. at 501 nm.





NanOZ Fluo LNP-DiI: is designed for the effective monitoring and imaging of intracellular trafficking and biodistribution of LNPs in vitro and in vivo respectively. It contains a lipophilic carbocyanine dye DiI having an excitation max. at 549 nm, and emission max. at 565 nm.

NanOZ Dual Fluo LNP (DiO/Cy5-mRNAFLuc): is designed for the effective monitoring and imaging of intracellular trafficking and biodistribution of LNPs in vitro and in vivo respectively. It contains a lipophilic carbocyanine dye DiO having an excitation max. at 487 nm, and emission max. at 501 nm and Cy5 mRNA having excitation max. at 651 nm, and emission max. at 670 nm.



#### LNP DIY

**NanOZ LNP-DIY** are designed for the development of LNP by customer at their ease by using different formulation methods at different N/P ratio. They are ready-to-use dried lipid mix at the total lipid concentration of 25 mM when reconstituted in 1mL ethanol, for LNP-mRNA, LNP-DNA, LNP-saRNA or LNP-sqRNA formulation.

NanOZ LNP-DIY (SM102): customers can produce themselves a monodisperse LNP encapsulating mRNA within the size range of 50 nm to 200 nm through microfluidic/ impingement jets mixing (IJM)/T-junction mixing technology with an encapsulation efficiency of 80-96 % under optimized conditions. One vial of 25 mM will allow the production of 4 formulations of 1 mL with a concentration of 100µg/mL of mRNA.

NanOZ LNP-DIY (FP105): customers can produce themselves a monodisperse LNP encapsulating mRNA within the size range of 50 nm to 200 nm through microfluidic/ impingement jets mixing (IJM)/T-junction mixing technology with an encapsulation efficiency of 80-96 % under optimized conditions. One vial of 25 mM will allow the production of 4 formulations of 1 mL with a concentration of 100µg/mL of mRNA.

NanOZ LNP-DIY (FP105s): customers can produce themselves a monodisperse LNP encapsulating siRNA within the size range of 50 nm to 200 nm through microfluidic/ impingement jets mixing (IJM)/T-junction mixing technology with an encapsulation efficiency of 80-96 % under optimized conditions. One vial of 25 mM will allow the production of 4 formulations of 1 mL with a concentration of  $100\mu g/mL$  of mRNA.

NanOZ LNP-DIY (SS1): customers can produce themselves a monodisperse LNP encapsulating mRNA / DNS / saRNA / sgRNA within the size range of 50 nm to 200 nm through microfluidic/ impingement jets mixing (IJM)/T-junction mixing technology with an encapsulation efficiency of 80-96 % under optimized conditions. One vial of 25 mM will allow the production of 4 formulations of 1 mL with a concentration of  $100\mu g/mL$  of mRNA.







Available in 0.5mL. 1mL and 5 mL



Cat. No. Cat. No. LFR10500 0,5mL (2\*250μL) of LNP-DiR LDIY102 Dried 25 mM lipid mix with SM102 0,5mL (2\*250µL) of LNP-DiO Dried 25 mM lipid mix with FP105 LFO10500 LDIY105 LFL10500 0.5mL (2\*250uL) of LNP-Dil LDIY10.5s Dried 25 mM lipid mix with FP10.5s 0,5mL (2\*250μL) of LNP-DiO with Cy5 mRNA F-Luc (100μg/mL) Dried 25 mM lipid mix with SS1

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# QUALITY CONTROL

We do know that the reproductibility of scientific experiments is very important to researchers. That is why we developed a quality control system tailored to each product, enabling us to detect defective batches immediately.

Our LNP formulations, whether custom or catalog, undergo standard quality control, including measurement of size and charge, encapsulation efficiency, sterility and RNA concentration. However, we have developed additional tests to extend the analysis to include endotoxin and mycoplasma detection, and lipid content measurement.

	Standart QC	Superior Grade QC*
Size	<b>✓</b>	<b>✓</b>
Charge	<b>V</b>	<b>✓</b>
Encapsulation efficiency	<b>✓</b>	<b>V</b>
Sterility	<b>✓</b>	<b>V</b>
RNA concentration	<b>V</b>	<b>V</b>
Endotoxin		<b>V</b>
Mycoplasma detection		<b>V</b>
Lipid content		<b>✓</b>

<sup>\*</sup>contact us to get a quote

# MRNA

Custom mRNA

Reporter gene mRNA

Fluorescent mRNA

Genome editing mRNA

Gene Replacement mRNA

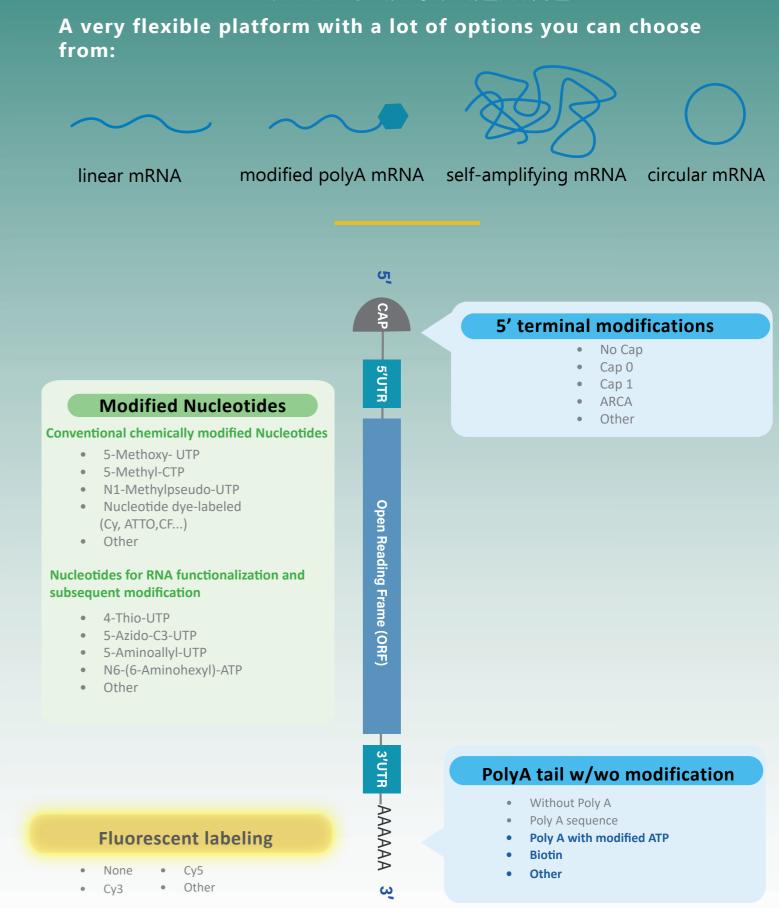
Interleukine mRNA

Vaccine / Antigen mRNA

Transcription Factor mRNA

mRNA Production Kits

# MRNA CUSTOM SERVICE



**Gene Synthesis** Send us your nucleotide or peptide sequence OR Send us your PCR product **Cloning DNA Template Preparation EXPERTISE DNA Sequencing** OR Send us your plasmid template **Plasmid DNA BIOSCIENCES** Linearization **RNA In-vitro Transcription Optional post-transcriptional modifications:**  Capping Poly-A • Biotin ... **Purification** Size, Integrity & Purity Fragment analyser Agarose gel **Quality Control** Concentration 260/280; 260/230 **Transfection / Expression \*** Open Reading Frame (ORF) 3'UTR —AAAAAA 3' We ship your fully custom-made mRNA in 6-8 weeks

Watch our video online!



Don't hesitate to ask for other options such as **concentration**, **buffer**, **desired** UTR, codon optimization etc...

# Reporter mRNAs

Reporter genes are commonly used in cell biology research . These capped (Cap1) and polyadenylated mRNAs are optimized for mammalian systems and are composed of unmodified NTPs or are modified (moU replaces U) to reduce immune stimulation. They mimic fully processed mature mRNAs.

### APPLICATION

 Reporter mRNAs can be used as controls to study transfection and expression in mammalian cells using a variety of assays.

**Tomato mRNA:** Designed to produce high expression level of Orange Fluorescent Protein. The produced Tomato has an exitation maximum at 551-557 nm and emission maximum at 579-583 nm.

**GFP mRNA:** Designed to produce high expression level of Green Fluorescent Protein. It is a commonly used direct detection reporter in mammalian cell culture, yielding bright green fluorescence with an exitation peak at 488 nm and an emission peak at 507 nm.

mCherry mRNA: Designed to produce the mCherry Fluorescent protein which is derived from DsRed, a protein found in Discosoma sp. mCherry is a monomeric fluorophore with an exitation peak at 587 nm and emission at 610 nm. mCherry is photostable and resistant to photobleaching.

**F-Luc mRNA:** Designed to produce high expression level of FireFly Luciferase. It is commonly used in mammalian cell culture to measure both gene expression and cell viability. FireFly Luciferase emits bioluminescence in the presence of its substrate: lucficerin.

**R-Luc mRNA:** Designed to produce high expression level of Renilla Luciferase. R-Luc mRNAs can be used as control of transfection efficiency as a reporter gene.

**B-Gal mRNA:** Designed to produce the enzyme encoded by the bacterial Lac Z gene. Beta-Gal catalyzes the conversion of Beta-galactosides into monosaccharides. It is a common marker gene used to assess transfection efficiency by measuring enzymatic activity after X-Gal staining or colorimetric assay (CPRG, ONPG kit).

# Fluorescent mRNAs

MAIN FEATURES

• Mature mRNAs

Capped (Cap1) and polyadenylated

Optimised for mammalian system

RECOMMENDED APPLICATION
Study of gene expression

and regulation

Cy5 GFP mRNA / Cy3 GFP mRNA: have been designed to produce high expression level of Green Fluorescent Protein. It can be used as control of transfection efficiency. When labeled with Cy5 or CY3, F-Luc mRNA can be easily traced to analyze mRNA delivery and translation efficiency.

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Cy5 F-Luc mRNA / Cy3 F-Luc mRNA / Alexa488 F-uc mRNA: have been designed to produce high expression level of FireFly Luciferase. It is commonly used in mammalian cell culture to measure both gene expression and cell viability. It emits bioluminescence in the presence of the substrate, luciferin. When labeled with Cy5, CY3 or Alexa488, F-Luc mRNA can be easily traced to analyze mRNA delivery and translation efficiency.

 Cat. No.
 Product

 MRNA9
 Tomato mRNA

 MRNA15
 GFP mRNA

 MRNA8
 mCherry mRNA

 MRNA16
 F-Luc mRNA

 MRNA20
 R-Luc mRNA

 MRNA17
 B-gal mRNA

Cat. No.
MRNA11b / MRNA15b / MRNA22b
MRNA11c / MRNA15c / MRNA22c
MRNA16a / MRNA12a / MRNA24b
MRNA16b / MRNA12b / MRNA24b
MRNA16c / MRNA12c / MRNA24b

Product Cy3 GFP mRNA Cy5 GFP mRNA Alexa488 F-Luc mRNA Cy3 F-Luc mRNA Cy5 F-Luc mRNA

Also available in 5moU and (N1-mψ) Available in 20 μg, 100 μg, 1mg

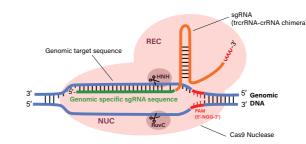
### APPLICATION

Genome editing mRNA are used to inroduce a variety of genetic alteration (deletio, insertion...) into

Cas9 endonuclease mRNA: Delivery of RNA encoding the Cas9 protein is an attractive non-viral method for introduction of CRISPR/Cas9 machinery into the cell. Unlike gene based delivery methods, mRNA based strategies are transient in function, leading to the eventual removal of the nuclease from the cell and circumventing the risks associated with integration into the host genome. The RNA -guided cas 9 endonuclease is used to induce site-directed double strand breaks in DNA. These breacks can lead to gene inactivation or introduction of heterologous genes, providing efficient tools for Genome editing.

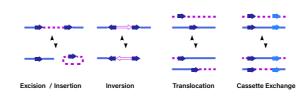
CRE Recombinase mRNA: CRE mRNA encodes for the CRE Recombinase. It is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between two loxP sites. Each recombinase catalyzes 4 types of DNA exchange reactions between short specific target sequences (30-40 nucleotides).

Cas13d mRNA: The Cas13d MRNAs encodes for the RNA-guided Cas13d endonuclease used to induce site-directed RNA degradation. Cas13d employs CRISPR-associated RNAs (crRNAs) that contain a customizable 22-nt spacer sequence that can direct the Cas13d protein to specific RNA molecules for targeted RNA degradation. The high catalytic activity of Cas13d in human cells provides a potential mechanism for targeting specific viral RNA genome degradation and viral gene expression inhibition.



mammalian cells with Genome editing techniques.

The CRISPR-Cas9 nuclease programmed with sgRNA



Cre recombinase induces 4 types of site-specific genome modifications

# RECOMMENDED APPLICATION introduction of genetic alteration into mammalian cells

# Gene Replacement mRNA

**EPO mRNA:** This mRNA encodes for the human Erythropoietin protein, an hormone that controls erythropoiesis. EPO acts as a hematopoietic growth factor and stimulates the synthesis of red blood cells in the bone marrow. EPO mRNA is commonly used for gene replacement and serves as model for expression of any secreted protein. Its expression can easily be evaluated by enzyme-linked immunosorbent assay (ELISA) while EPO's effect on red blood cell production is detected by measuring reticulocyte levels and the hematocrit from whole blood using a hematocrit assay. Measurement of EPO are rather straightforward and well established.

### Interleukine mRNA

h IL2 mRNA: Designed to produce high expression level of Interleukin-2 (IL-2) proteins.

Cat. No.	Product	Cat. No.	Product
MRNA19-20	EPO mRNA (5moU) 20 μg	MRNA30-20	Cas9 mRNA 20 µg
MRNA18-20	Unmodified EPO mRNA 20 μg	MRNA31-20	Cas9 mRNA (5moU) 20 µg
MRNA4-20	EPO mRNA (N1-mψ) 20 μg	MRNA25-20	Cas9 mRNA (N1-m <b>ψ</b> ) 20 µg
MRNA55-20	h IL2 mRNA 20 µg	MRNA33-20	CRE mRNA Unmodified 20 µg
MRNA56-20	hl L2 mRNA (5moU) 20 μg	MRNA32-20	CRE mRNA (5moU) 20 µg
MRNA57-20	h IL2 mRNA (N1-m $\psi$ ) 20 µg	MRNA26-20	CRE mRNA (N1-m $\psi$ ) 20 $\mu$ g
		MRNA27-20	Cas13d mRNA 20 µg
		MRNA28-20	Cas13d mRNA (5moU) 20 µg
		MRNA29-20	Cas13d mRNA (N1-m <b>ψ</b> ) 20 μ

Available in 20 μg, 100 μg, 1mg

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# Nucleic acid Vaccine combines the positive features of live attenuated vaccines while avoiding many potential safety limitations. These vaccines present several advantages over conventional vaccines such as:

- Mimicking a live infection by expressing antigens in situ after immunization and priming both B and T cell responses including cytotoxic T lymphocytes.
- Revealing focused immune responses directed toward the selected antigens of interest with no pot-ential reversion to pathogenicity.
- Serving the dual purpose of expressing the desired antigen as well as acting as an adjuvant.
- Having a superior safety profile compared to inactivated viruses or pathogenss or are modified (moU replaces U) to reduce immune stimulation. they mimic fully processed mature mRNAs.

### MRNA VACCINES BENEFITS



mRNA vaccines present a better safety profile than DNA vaccines: DNA vaccines display a long term expression, a potential risk for genome integration and induction of anti-DNA antibodies. The main advantages of mRNA vaccines come from the intrinsic properties of the mRNA:

- They are produced using cell-free enzymatic transcription.
- The transient expression of mRNA encoded antigen enables a more controlled antigen espression
- and minimizes the risk of tolerance induction that can be associated with long-term exposure.
- There is thus an absence of any additional encoded protein which exclude the possibility of raising
- undesired immune response or interaction with the host.  $\!7$
- Their stabilized design allows higher level of expression in vivo.

### MAIN FEATURES

- Mature mRNAs
- Capped (Cap1) and polyadenylated
- Optimised to yield improved stability and performance



OVA mRNA: Designed to produce high expression level of Ovalbumin Protein.

**Spike SARS-CoV-2 mRNA:** Designed to produce high expression level of Spike Protein of SARSCOV-2 virus.

N SARS-CoV-2 mRNA: Designed to produce high expression level of the nucleocapsid protein of SARS-CoV-2 virus

**Spike DELTA mRNA**: Designed to produce high expression level of DELTA Mutant Spike Protein of SARS-CoV-2 virus.

**Spike OMICRON mRNA**: Designed to produce high expression level of OMICRON Mutant Spike Protein of SARS-CoV-2 virus

**HA-H1N1** mRNA: Designed to produce high expression level of the hemagglutin surface glycoproteins of H1N1 subtype of Influenza A virus.

**HA-H3N2** mRNA: Designed to produce high expression level of the hemagglutin surface glycoproteins of H3N2 subtype of Influenza A virus.

Cat. No.	Product	Cat. No.	Product
MRNA41-20	OVA mRNA (5moU) 20 μg	MRNA 39-20	Spike Omicron mRNA (5moU) 20 μg
MRMRNA42-20	Unmodified OVA mRNA 20 μg	MRNA 38-20	Unmodified Spike Omicron mRNA 20 µg
MRNA 40-20	OVA mRNA (Ni-mψ) 20 μg	MRNA 44-20	Spike Omicron mRNA (N1-mψ) 20 μg
MRNA 35-20	Spike SARS-CoV-2 mRNA (5moU) 20 µg	MRNA 37-20	Spike SARS CoV-2 DELTA mRNA (5moU) 20 µg
MRNA 34-20	Unmodified Spike SARS-CoV-2 mRNA 20 µg	MRNA 36-20	Unmodified Spike SARS CoV-2 DELTA mRNA 20 µg
MRNA 43-20	Spike SARS-CoV-2 mRNA (N1-mψ) 20 μg	MRNA 45-20	Spike SARS CoV-2 DELTA mRNA (N1-mψ) 20 μg
MRNA 52-20	N SARS-CoV-2 mRNA (5moU) 20 µg	MRNA 46-20	HA-H1N1 mRNA (5moU) 20 µg
MRNA 53-20	Unmodified N SARS-CoV-2 mRNA 20 µg	MRNA 47-20	Unmodified HA-H1N1 mRNA 20 µg
MRNA 54-20	N SARS-CoV-2 mRNA (N1-mψ) 20 μg	MRNA 48-20	SHA-H1N1 mRNA (N1-mψ) 20 μg
		MRNA 49-20	HA-H3N2 mRNA (5moU) 20 µg
		MRNA 50-20	Unmodified HA-H3N2 mRNA 20 µg
		MRNA 51-20	HA-H3N2 mRNA (N1-mψ) 20 μg
	Available in 3	<sup>2</sup> O µg, 100 µg, 1mg	17- 10

# Transcription Factor mRNA

**c-Myc mRNA:** has been designed to produce high expression level of c-Myc protein. It belongs to the basic Helix-Loop-Helix (bHLH) family of proteins which are transcription factors that play important roles during the development of various metazoans. They are also involved in human diseases, particularly in cancerogenesis.

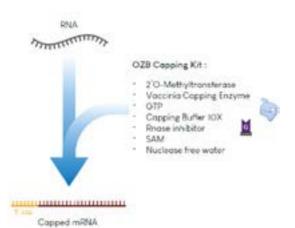
h. SOX2 mRNA: has been designed to produce high expression level of Sox2 protein. SRY-box transcription factor 2 (Sox2) gene encodes a member of the SRY-related HMG-box (SOX) family of transcription factors involved in the regulation of embryonic development and in the determination of cell fate. The product of this gene is critical for early embryogenesis and for embryonic stem cell pluripotency.

### mRNA Production Kits

IVT Kit: This kit is designed for the in vitro transcription (IVT) of your linear plasmid DNA or PCR fragment using the T7 polymerase. It is supplied with enough components for 25 reactions (20µl each), giving a yield of 2.5 mg of RNA per kit.

### Key benefits:

- High-yield RNA production
- Efficient polymerase (even with modified nucleotides)
- Separeted NTPs for an easier optimization / replacement with modified nucleotids



Poly(A) Kit: This kit is intended for the enzymaticaddition of a poly(A) tail to the 3-hydroxyl termini of capped or non-capped RNAs independently of the template. The Poly(A) Tailing Kit allows the addition of approximately 200 nucleotides per reaction (based on 100µg of RNA).

### Key benefits:

- Highly processive enzyme
- Excellent reproducibility
- Quick and easy to use

Cat. No. Product

MRNA58-20 c-Myc mRNA (unmodified) 20 µg

MRNA59-20 c-Myc mRNA (5moU) 20 µg

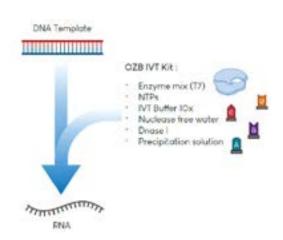
MRNA60-20 c-Myc mRNA (NI-mψ) 20 µg

MRNA61-20 SOX2 mRNA (unmodified) 20 µg

MRNA62-20 SOX2 mRNA (5moU) 20 µg

MRNA63-20 SOX2 mRNA (NI-mψ) 20 µg

Available in 20 μg, 100 μg, 1mg



Capping Kit: This kit is dedicated to the in vitro enzymatic capping of mRNA. It permits the addition of a Cap0 or Cap1 structure on the 5' terminus of mRNA, improving its stability and translation.

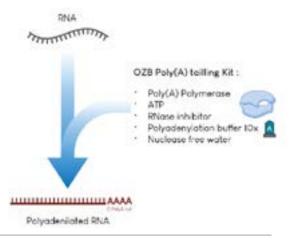
### Key benefits:

- Great capping efficiency
- Quick reaction
- Easy steps

Cat. No. IVT-K25

CAP-K25

POLYA-K25



Product IVT Kit (25 reactions) Capping Kit (25 réactions) Poly(A) Kit (25 reactions)

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# QUALITY CONTROL

We do know that the reproductibility of scientific experiments is very important to researchers. That is why we developed a quality control system tailored to each product, enabling us to detect defective batches immediately.

Our mRNAs, whether custom or catalog, undergo standard quality control, including measurement of size, integrity and purity by fragment analyzer, agarose gel and NanoDrop (concentration 260/280 and 260/230), a sterility test and an experimental validation (transfection / expression). However, we have developed additional tests to extend the analysis to include endotoxin detection and double strand RNA (dsRNA quantification.

	Standart QC	Superior Grade QC*
Fragment analyzer	<b>✓</b>	<b>✓</b>
Agarose gel	<b>V</b>	<b>V</b>
Concentration (260/280 & 260/230)	<b>✓</b>	<b>✓</b>
Sterility	<b>✓</b>	<b>✓</b>
Transfection / Expression	<b>V</b>	<b>✓</b>
Endotoxin		<b>✓</b>
dsRNA		<b>✓</b>

<sup>\*</sup>contact us to get a quote

# EIN DELIVERY

Ab-DeliverIN™

Pro-DeliverIN™

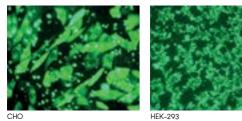
Pro-DeliverIN™ CRISPR

# Ab-DeliverIN<sup>TM</sup>- Antibody delivery system

Ab-DeliverIN<sup>TM</sup>, a lipid-based reagent, is the sole serum compatible agent allowing the delivery of functional antibodies into living cells. Due to its unique properties, Ab-DeliverIN $^{\text{TM}}$  forms non-covalent complexes with antibodies trought electrostatic and hydrophobic interactions. Chemicals or genetic couplings are not necessary. In addition, delivered antibodies retain their structure and function so that the antibodies transported in cells are functional and can reach their intracellular target.

▶ To learn more about Lipofection Technology see page 14

Intracellular delivery of fluorescently labeled IgG into various cells



Fluorescently labeled polyclonal IgG from human serum was delivered into cells seeded in a 24-well plate using 1 µg FITC-IgG and 2 µL Ab-DeliverIN per well After 24H, cells were fixed and observed under fluore

### MAIN FEATURES

DELINERY

PROTEIN

- Intracellular delivery of functionally active antibody
- Highly efficient in primary cells & cell lines Delivery of antibodies in a large number of immortalized and primary cells including RAW 264.7, NIH-3T3, primary neurons and glial cells...

Highest efficiencies can be achieved in less than 5 hours

### • Serum compatible

Significant amount of antibody transported with no medium change required

### • Biodegradable & non-toxic

Ab-DeliverIN™ does not interfere with cellular mechanisms

# Time course of intracellular antibody delivery ►% fluorescent cells Amount of protein/cells 4H 3H Incubation Time

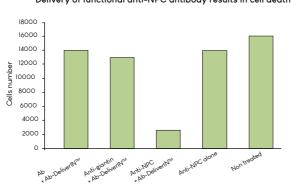
1  $\mu g$  of FITC-labeled antibody was delivered into NIH3T3 cells. Cells were collected and fixed with 2% PFA at the indicated time point. The number of fluorescent cells and the mean of fluorescence were determined by cytofluorimetry. The mean fluorescence was used to evaluate the amoun

### **APPLICATIONS**

- · Suitable for all kinds of antibodies
- · Efficient on primary cells & cell lines
- Ideal for delivery applications: intracellular localization studies in living cells, protein function with blocking antibodies, protein interaction blocking, FRET studies...

RECOMMENDED APPLICATION Intracellular delivery of antibodies

Delivery of functional anti-NPC antibody results in cell death



### **PUBLICATIONS**

"HIV is inactivated after transepthelial migration via adult oral epithelial cells but not fetal epithelial cells".

Tugizov S.M et al - Virology. 2011

"Antibody delivery into viable epimastigotes of Trypanosoma cruzi as a tool to study the parasite biology".

Acosta-Viana K.Y et al - Adv Biosc Biotech. 2013

"Apical transport of influenza A virus ribonucleoprotein requires Rab11-positive recycling endo-

Momose F. et al - PLoS One. 2011

▶ Browse our citation database online

### Ab-Deliverin™ is provided with 100 µL of FITC labeled IgG Postive control.

Cat. No.	Product	No. of assays
Al20100	Ab-DeliverIN 100 μL	50-100
Al20250	Ab-DeliverIN 250 μL	125-250
Al20500	Ab-DeliverIN 500 μL	250-500
Al21000	Ab-DeliverIN 1 mL	500-1000

# Pro-DeliverIN<sup>TM</sup>- Protein delivery reagent

Pro-DeliverIN™, a lipid-based reagent, is the first serum compatible agent to deliver functional proteins into living cells. Due to its specific properties, it is able to capture proteins through electrostatic and hydrophobic interactions. Consequently, there are no needs of covalent linking (chemical or genetic). The proteins delivered inside cells retain their structure and function.

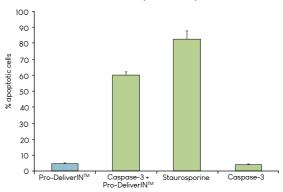
▶ To learn more about Lipofection Technology see page 14

### **APPLICATIONS**

- Suitable for primary cells & cell lines
- Ideal for protein delivery applications: intracellular localization studies in living cells, protein - protein interaction, FRET studies...

RECOMMENDED APPLICATION Intracellular delivery of proteins

### Pro-DeliverIN delivers fully functional proteins



HeLa cells were treated with 15 ng of active human caspase-3 & 5  $\mu$ L of Pro-DeliverIN $^{\rm IM}$  reagent in 24-well plates. As controls, cells were treated either with 15 ng of caspase–3 alone, 5  $\mu$ L of Pro–DeliverIN<sup>TM</sup> alone or 100 nM staurosporine (positive control). After 7H of incubation, cells were stained with Annexin-FITC and propidium iodide. Apoptotic and dead cells were monitored by cytofluorimetry.

### **PUBLICATIONS**

Discover how to use Pro-DeliverIN for nanobodies delivery in HeLa and A549

"A peptide tag-specific nanobody enables high-quality labeling for dSTROM imaging".

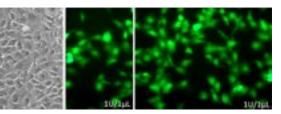
Virant D. et al - Nature Communications. 2018

"Non-Viral Generation of Neural Precursor - like Cells from Adult Human Fibroblasts".

Maucksch C. et al - J Stem Cell Reg Med. 2013

▶ Browse our citation database online

### Pro-DeliverIN™ succeeded in transporting active CRE recombinase



Homologous Recombination: HEK-293 cells were stably transfected with GFF plasmid containing LOX sequences. Active CRE recombinase Enzyme was delivered using Pro-DeliverIN™ reagent & GFP-expression was monitored 96H after

### MAIN FEATURES

- Intracellular delivery of functionally active proteins
- Highly efficient in primary cells & cell lines Proteins are efficiently delivered in the cytoplasm of a large number of living cells (3T6, A549, COS-1, HaCat, HeLa, Jurkat, L929, MDCK, N2A, U87...) including primary cells
- Fast delivery

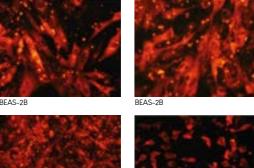
The proteins are transported inside cells in 3 to 4 hours

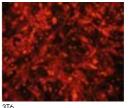
• Serum compatible

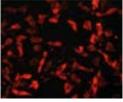
No medium change required

· Biodegradable and high cell viability

### Intracellular delivery of R-phycoerythrin into various cell lines







1 µg of R-Phycoerythrin was mixed with 2 µL of Pro-DeliverIN™ reagent implexes were then incubated with different cell lines in 24-well plates. Living cells were observed 24H later by fluorescence microscopy

### Pro-DeliverIN™ is provided with 100 µL of R-Phycoerythrin Positive Control.

Cat. No.	Product	No. of assays
PI10100	Pro-DeliverIN 100 μL	50-100
PI10250	Pro-DeliverIN 250 μL	125-250
PI10500	Pro-DeliverIN 500 μL	250-500
PI11000	Pro-DeliverIN 1 mL	500-1000

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# Pro-DeliverIN™ CRISPR

Pro-DeliverIN™ CRISPR is a transfection reagent optimized for recombinant Cas9 protein delivery or Cas9/gRNA RNP complexes. For your gene editing applications, this reagent provides high transfection efficiency with minimal toxicity.

### Why choose Cas9 protein instead of Cas9 DNA or mRNA?

The Cas9 recombinant protein is delivered more rapidly than nucleic acids and is fully active once inside the cells without latency period (in contrast to transcription and translation machineries required for the nucleic acids).

### **FOCUS ON**

Efficient delivery represents a critical step for genome editing experiments.

To maximize your results using Pro-DeliverIN CRISPR, we developed our Optimized Cas9 nuclease (see hereunder).

Designed with a genuine targeting sequence, this Cas9 contruct is more efficient at targeting the Cas9 protein to the nucleus.

•A Specific CRISPR/Cas9 Delivery Kit is also available

Pro-DeliverIN™ CRISPR is provided with 100µL of R-Phycoerythrin Positive Control.

### Cat. No. PIC60100 PIC60500

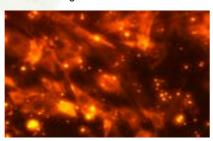
Pro-DeliverIN CRISPR 100 μL Pro-DeliverIN CRISPR 500 µL

### MAIN FEATURES

- High efficiency delivery of Cas9 protein or Cas9 gRNA complexes
- Low cell toxicity

Enables to start your experiment with less cells

High delivery of Phycoerythrin recombinant protein in MEF cells using Pro-DeliverIN CRISPR



MEF cells were plated in 24-well plates in complete medium at the optimum density of 50-70% confluence at the time of ransfection. The PE was monitored 24H after transfection by

# **Optimized Cas9 Nuclease**

Optimized Cas9 Nuclease S. Pyogenes is designed for **genome editing** in living cells or organisms and also for in vitro digestion.

No. of assays

50-100

125-250

### Increased genome editing efficiency using Cas9/RNP delivery:

Successful CRISPR/Cas9 genome editing can be performed through diverse approaches (plasmids, mRNA, nuclease, viral delivery).

### Why choose Cas9 protein instead of Cas9 DNA or mRNA?

The Cas9 recombinant protein is delivered more rapidly than nucleic acid and is fully active once inside the cells without latency period (in contrast to transcription and translation machineries required for the nucleic acids). These features make nuclease protein delivery particularly well suited for precision genome engineering.

To learn more about CRISPR Cas9 genome editing see page 16

### The Cas9 nuclease programmed with sgRNA

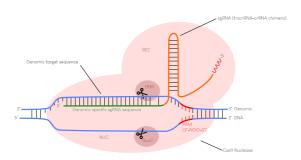
### **FOCUS ON**

Efficient nucleic acids and/or enzymes delivery represents a critical step for genome editing experiments.

For the most efficient Cas9 nuclease delivery, we recommend Pro-DeliverIN™ CRISPR transfection reagent.

### RECOMMENDED APPLICATION

For CRISPR Cas9 genome editing experiments



Upon binding, the short guide RNA (sgRNA) specifically targets a short DNA sequence-tag (PAM). Cas9 nuclease cleaves DNA three nucleotides upstream the

Cat. No.	Product	Description
CAS9050	Cas9 Nuclease 50 µg	1 mg/mL
CAS9100	Cas9 Nuclease 100 µg	1 mg/mL
CAS9500	Cas9 Nuclease 500 µg	1 mg/mL
CAS9PIC	Cas9 Nuclease Special CRISPR/Cas9 Delivery Kit	50 μg Cas9 Nuclease + 100 μL of Pro-DeliverIN CRISPR

### Adjuvants for protein based vaccines

Liposome-based Adjuvants

Aluminium Gels

Squalene Emulsion

Freund's Adjuvants

### Adjuvants for mRNA & DNA based vaccines

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# Aluminum gels

Calivax-DotaP is a cationic lipid-based composition for liposome-mediated mRNA, DNA or protein vaccines.

DOTAP (N-[1-(2,3-Dioleoyloxy)propyl]-N,N,N trimethyl-ammonium methyl-sulfate):Cholesterol (1:1 molar ratio) cationic lipid composing this genetic adjuvant allows the formation of complexes with mRNA, plasmid DNA or antigen protein to form an efficient lipoplexes-based nanoparticle delivery system (LPD).

Compatible with most immunization procedures such as intramuscular, intraepidermal, intravenous, intraperitoneal or subcutaneous.

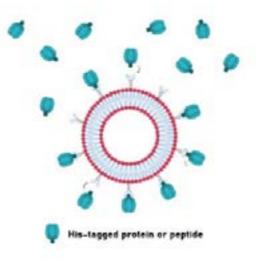
RECOMMENDED APPLICATION
Vaccine Carrier

# Surface Reactive Liposome

<u>LipoVax NTA(Ni)</u> is Ni2+-based liposome adjuvant that can anchor diverse histidine-tagged proteins or peptides to an antigen-presenting carrier to trigger immune response (immunization, vaccination, antibody generation).

- Contains phospholipid, cholesterol and nickel-chelating lipid (60:39:1), showing high affinity to bond with electron-rich ligand such as histidine. Therefore, complexes can be generated using Ni-nitrilotriacetic acid (NTA) and his-tagged proteins or peptides. The resulting liposomes are non-viral biologics delivery systems, self-assembled from metal-chelating lipid and his-tagged immunogens such as envelope glycoproteins.
- Compatible with most immunization procedures such as intramuscular, intraepidermal, intravenous, intraperitoneal or subcutaneous.

RECOMMENDED APPLICATION
His-Tagged-based Protein or Peptide Carrier Vaccine Adjuvant.



Cat. No. CV02000 Product CaLiVax-DOTAP 2 mL Cat. No. LV02000 Product

Also available in bulk, please contact us for a quote

Also available in bulk, please contact us for a quote

Aluminum Gels are the most common adjuvants used in approved prophylactic vaccines because of their excellent safety profile and ability to enhance protective humoral immune response. It has been observed that aluminium compounds act by a depot effect and also by direct activation of the immune cells. Adsorption or entrapment of antigens in aggregates through hydrophobic and electrostatic interactions favors a high local antigen concentration and improved uptake by antigen presentating cells (APC).

### Two classical aluminum-based adjuvants:

RECOMMENDED APPLICATION Stimulation of Th2 response, Antibody production **AlumVax Hydroxide:** a crystalline aluminum oxyhydroxide that is positively charged at physiological pH (pI=11), suitable for adsorption of negatively charged acidic proteins (such as albumin).

**AlumVax Phosphate:** an amorphous aluminum hydroxyphosphate which is negatively charged at physiological pH (pI=5–7), suitable for adsorption of positively charged or neutral, alkaline proteins

# Freund's adjuvants

Freund's Adjuvants consist of a mixture of mineral oil and emulsifier in a ratio of 85% v/v oil and 15% v/v emulsifier. Importantly, the Freud's Adjuvants are not a pre-formed emulsion and must be mixed with an equal volume of aqueous solution of antigen and subsequently emulsified prior to use.

### Two Freund's adjuvants available:

**CFAVax** (Complete Freund's Adjuvant) is a water-in-oil emulsion containing 1 mg per mL heat-killed.

**IFAVax** (Incomplete Freund's Adjuvant) is a waterin-oil emulsion without addition of heat-killed mycobacteria (*Mycobacterum butyricum*) RECOMMENDED APPLICATION
Stimulation of Th1 (CFAVax) and
Th2 (IFAVax) responses
Initial immunization, Antibody production

# Squalene emulsion

**SqualVax** is an oil-in-water emulsion made of squalene droplets in a continuous aqueous phase. It is fully biodegradable and has long term persistence in the organisms. The emulsion acts more specifically on macrophages present at the site of injection. This formulation enhances the immune response and the differentiation of monocytes towards a mature phenotype, thereby promoting migration of antigen-loaded cells to the draining lymph node.

### RECOMMENDED APPLICATION

Stimulation of Th2 response, preferentially with balanced Th1/Th2 cell phenotype



Cat. No.	Product	Cat. No.	Product
AH0050	AlumVax Hydroxide 50 mL	AP0050	AlumVax Phosphate 50 mL
AH0250	AlumVax Hydroxide 250 mL	AP0250	AlumVax Phosphate 250 mL
IFA0010	IFAVax 10mL	CFA0010	CFAVax 10mL
IFA0050	IFAVax 5x10mL	CFA0050	CFAVax 5x10mL
IFA0100	IFAVax 10x10mL	CFA0100	CFAVax 10x10mL
SQ0050	SqualVax 5x10mL	SQ0100	SqualVax 10x10mL
Also available	in bulk, please contact us for a auote		•

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# **CNE-CPO**

CNE-CPO is an oil-in-water Cationic Nano Emulsion made of squalene droplets and cationic polymers in a continuous aqueous phase.

The cationic components that compose this nano-emulsion make it a genetic adjuvant that allows the association with mRNA & plasmid DNA to form an efficient nanoparticle delivery system (NPD). NPD are non-viral gene delivery systems, self-assembled from cationic entities and negatively charged immunogen that function as vaccine carrier.

- Is biodegradable, an important advantage over alternative oils that have been used in emulsion adjuvants, like Freund's adjuvant that contains mineral oil (paraffin oil) and has long term persistence in the organism.
- CNE induces local stimulation and recruitment of DCs and granulocytes, differentiation of monocytes into DCs and increased uptake of antigen by DCs.
- Acts more specifically on macrophages present at the site of injection. A local increase of chemokines released also influences the recruitment of immune cells from the blood to the site of vaccination, creating an amplification loop.
- Enhances differentiation of monocytes towards a mature phenotype, thereby promoting migration of antigen-loaded cells to the draining lymph node.



# PolyVax-CPO

PolyVax-CPO (Cationic POlymer-based) is a cationic polymer genetic adjuvant that associates with mRNA & plasmid DNA to form an efficient polymer-based nanoparticle delivery system (NPD).

• Compatible with most immunization procedures: such as intramuscular, intraepidermal, intravenous, intraperitoneal or subcutaneous



RECOMMENDED APPLICATION Stimulation of Th1 (CFAVax) and

Th2 (IFAVax) responses
Initial immunization, Antibody production

 Cat. No.
 Product

 CNE1000
 CNE-CPO 1mL

 CNE5000
 CNE-CPO 5mL

Also available in bulk, please contact us for a quote

Cat. No. CP02000 Product PolyVax-CPO 2 mL

Also available in bulk, please contact us for a quote

# CELLULAR ASSAY KITS

### **Protein Quantification Assays**

FluoProdige Protein Assay Ki

Bradford Protein Assav Kit

BCA-PAK Protein Assay Ki

### Enzyme Detection - Reporter Gene Assays

B-Galactosidase & X-Gal Kits

SEAP Assav Ki

Luciferase Assay Ki

CPRG Kit

ONPG Kit

### Viability/Apoptosis/Stress Assays

ROS Detection Assay Kit

OZBlue Cell Viability Kit (Resazurin

MTT Cell Proliferation Assav Kit

Cellular Senescence Ki

WS1-8 Cell Proliferation Kit

Nitric Oxide Detection Kit

LDH Cvtotoxicity Assav Kit

# FluoProdige Assay Kit

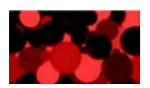
### Facilitates and Improves Protein and Peptide Quantification

The FluoProdige Protein Quantification Assay Kit presents a complete fluorometric assay for protein and peptide quantification.

This Assay Kit uses a **stable analogue to epicocconone** molecule that reversibly binds to Lysine, Arginine and Histidine residues in proteins and peptides to yield an intense red-fluorescent product. The fluorescent signal (~518/605) is directly proportional to protein amount among a wide range of protein concentration, rendering this kit highly sensitive.

### RECOMMENDED APPLICATION

For fluorescent measurement of protein amount Ideal for low amount of proteins and peptides



### MAIN FEATURES

• Accurate & highly sensitive

Detect as little as  $40\ ng/mL$  - Large linear detection range: Over 3-orders of magnitude

• Simple & fast
Protocol: 15min - Pic of signal within 30min - Signal
duration: up to 4 hours

• Safe & biodegradable
A natural product: non-toxic, heavy metal free

# Bradford Protein Assay Kit

Simply process your assay in a few minutes

The **Bradford Pak** is a straightforward and rapid Kit for determining the concentration of proteins in solution. This ready-to-use Kit is provided with 1X reagent (500mL) & 2 sets of BSA standards wich means that no dilution, filtration or calculation are required. It is based on the bilding of Coomassie Brilliant Blue G-250 dye to the proteins and particularly basic and aromatic amino acids residues.

### MAIN FEATURES

- $\bullet$  Accurate determination of protein concentration
- Ready-to-use prediluted standard protein
- Convenient packaging



# **BCA-PAK Protein Assay Kit**

### Robust and detergent-tolerant colorimetric detection

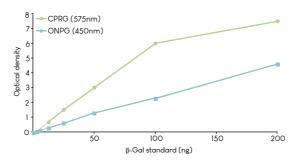
The BCA Kit is useful for colorimetric detection and quantification of total protein content even in the presence of detergents. It is based on the reduction of Cu2+ to Cu1+ by proteins in alkaline solution. Bicinchoninic acid (BCA) chelates with the reduced copper Cu1+ and forms a water-soluble purple reaction complex that exhibits a strong absorbance at 562 nm. Absorbance is linear over a wide range of protein concentrations between  $25\text{-}2000 \,\mu\text{g/mL}$ .

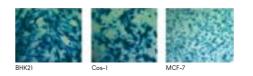
Cat. No.	Description	No. of assays
FPRO200	FluoProdige Assay Kit	2000 assays
BA00100	Bradford Pak	3570 to 5000 assays
BA00050	Bradford Reagent (500 mL)	3570 to 5000 assays
BA00070	BSA standard (2 sets)	7 vials of 2 mL
BCA2500	BCA-PAK Protein Assav Kit	2500 assays

# **β-Galactosidase & X-Gal Kits**

### Monitoring & Determination of lac Z transfected cells

LacZ is one of the most frequently reporter gene used in transfection experiments. The LacZ encoded protein beta-Galactosidase ( $\beta$ -Gal) is very stable, resistant to proteolytic degradation and easily tested.





### **FOCUS ON**

### Measuring high expression level of β-Gal Choose the ONPG Assay Kit

The levels of active  $\beta$ -Gal expression can be quickly measured by its catalytic hydrolysis of ONPG (o-nitrophenyl- $\beta$ -D-galactopyranoside) substrate to a bright yellow product (Absorbance at 405-420nm).

### Measuring low expression level of β-Gal

Choose the CPRG Assay Kit The high sensitivity of this substrate improves the measurement of  $\beta$ -Galactosidase activity when the reporter gene expression is low. (Absorbance

at 570-595 nm).

• Visualization of LacZ transected cells in vitro & in vivo Choose the X-Gal Staining Kit

It allows to visualize  $\beta$ -Gal expression through hydrolysis of the X-Gal substrate (5-bromo-4-chloro-3-indoyl- $\beta$ -D-Galactopyranoside) yielding blue precipitates.

# SEAP Assay Kit

### Reporter Gene Expression

**SEAP Assay Kit** (Secreted Alkaline Phosphatase) is a colorimetric assay for sensitive quantification of SEAP in culture medium from transfected cells or tissues.





- Colorimetric measure with a standard spectrophotometer or an ELISA reader (405nm)
- Mutiple samples can be analyzed in low volumes
- Convenient & economical packaging: no tablet, no powder

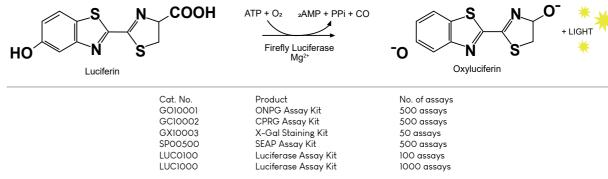


# Luciferase Assay Kit

Enzyme Detection – Reporter Gene Assays

### Monitoring Luciferase activity in transfected cells and tissues

The **Luciferase Assay Kit** is the most sensitive analytical tools for measuring gene expression. Fast and easy, accurate and linear, this Kit detects and quantify firefly luciferase in transfected eukaryotic cells reaching high sensitivity level



# **ROS Detection Assay Kit**

### Quantify cellular Reactive Oxygen Species (ROS)

Optimum levels of ROS play an important role in signaling pathways. However when ROS production increases and overhelms the cellular antioxidant capacity, it can induce macromolecular damage (by reacting with DNA, proteins and lipids) and disrupt thiol redox circuits. This damage can lead to apoptosis or necrosis.



**Ideal** for fluorescence microscopy microplate titration & cytometry

**Compatible** with adherent cells as well as suspension cells



The ROS Detection Assay Kit uses the cell-permeable fluorogenic probe 2', 7' Dichlorodihydrofluorescein diacetate (DCF-DA). Once DCF-DA has diffused into cells, it is deacetylated by cellular esterases to a non-fluorescent compound and rapidly oxidized by ROS into DCF. DCF is highly fluorescent and can be detected by microscopy, titration in microplate and also cytometry (ex: 485/em 535).

# OZBlue Cell Viability Kit (Resazurin)

### Check Viability without killing your cells!

A ready-to-use assay system based on fluorimetric/colorimetric quantification of metabolic activity in living cells. The Resazurin dye is used as an indicator of cell viability.



### MAIN FEATURES

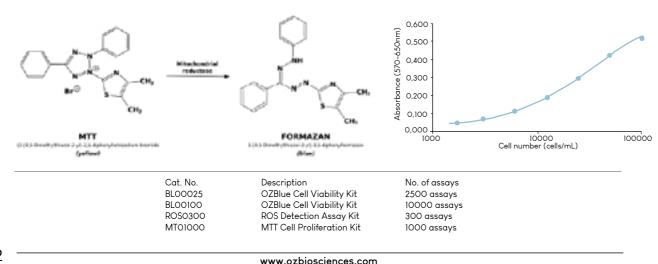
- Non-toxic OZBlue allows additional analysis on proliferating cells (mRNA, cytogenetic, apoptosis, immunophenotyping...) and continuous monitoring of cultures over time
- **Highly sensitive** An improved altenative to [³H]Thymidine incorporation and Tetrazolium reduction Assay (MTT, XTT)
- Simple A single ready-to-use reagent: no need of washing or extraction procedures

# MTT Cell Proliferation

### Colorimetric Plate Readout

MTT Cell Proliferation Assay Kit is designed for spectrophotometric quantification of cells growth, viability and proliferation and can be used as a direct indicator of cytotoxicity (such as for screening anticancer drugs) and apoptosis.

The MTT Cell Proliferation Kit is a colorimetric assay for measuring the mitochondrial reductases activity in living cells. It is based on the cleavage of membrane-permeable yellow tetrazolium salt MTT to blue/purple formazan crystals by metabolically active cells. This MTT assay kit contains sufficient reagents to perform 1000 assays in 96-well plate format.



## Cellular Senescence Kit

### Specific Stem Cells application

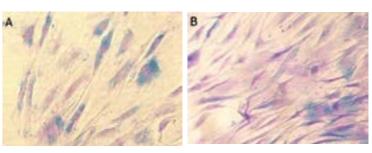
Ideal when cells in culture showed abnormalities typical of the Hayflick model - after a long period of normal growth - **Cellular Senescence Kit** provides an easy-to-use & efficient method to determine cellular senescence.

During senescence in mammalian cells, an endogenous lysosomal  $\beta$ -Galactosidase is over-expressed and is accumulated within the cells. The presence of this  $\beta$ -Gal activity is a marker of aging cell population *in vitro*.



- · Simple and Rapid
- · Ready-to-use
- Economical





Adipose derived Stem Cells at passage 7 (A) and Mesenchymal Stem Cells at passage 6 (B) stained with the Cellular Senescence Kit Nuclei counterstained with Crystal Violet solution, *not supplied*.

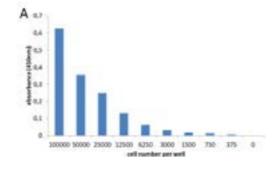
# WST8

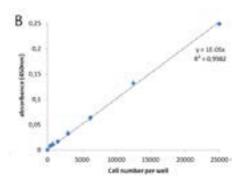
### Determination of viable cell number

The WST-8 Cell Proliferation Kit is a colorimetric assay for the determination of viable cell number and for studying induction or inhibition of cell proliferation in vitro.

### MAIN FEATURES

- More sensitive than other formazan-based assays
- Colorimetric measurement at 450 nm allows quantification of viable cells. Large linearity range independent from temperature, pH or concentration
- Rapid and ready-to-use. One step procedure / One reagent only. No need of washing or extraction procedures, no other solvent or solubilisation solution required
- **Highly reproducible.** Adaptable from 384-well plates for screening to larger cell culture formats / Perfect for High Throughput Screening
- Suitable for adherent as well as suspension cells. Compatible with cell lines, primary cells, bacteria...
- · Allow to perform real-time assays
- Final compound is not harmful for the cell





### Cell number titration

(A) Jurkat T cells were seeded at different concentrations in a 96-well plate and incubated 2h with WST-8 Cell Proliferation Kit.

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(B) Linearity between absorbance and cell number varies according to the cell type and metabolism

Cat. No. Description
GXS0003 Cellular senescence kit (Fixing Buffer: 125 mL, 10X PBS: 75 mL,
Staining Buffer: 125 mL, 25X Stock Solution of SA X-Gal: 4 x 1 mL)
WS1000 10 mL of WST-8 reagent

No. of assays 50 assays

1000 assays

92

Enzyme Detection – Reporter Gene Assays

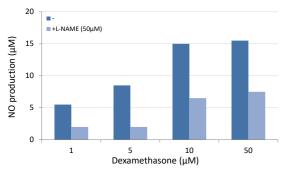
# Nitric Oxide Detection Kit

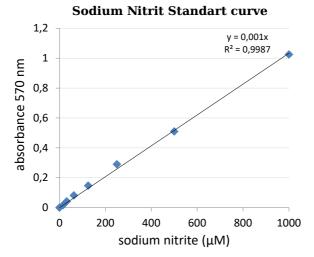
### Colorimetric Plate readout

The **Nitric Oxide Detection Kit** is a colorimetric assay for the indirect determination of Nitric Oxide concentrations based on the enzymatic conversion of nitrate to nitrite by a nitrate reductase.

NO are important physiological messengers and effector molecules involved in many physiological processes such as vasodilation, inflammation, thrombosis or immunity (...). This assay is based on the reaction originally described by Griess to detect NO2– in a variety of biological fluids such as cell culture medium, plasma, serum, or urine.

### **Dexamethasone effect on NO production**





### MAIN FEATURES

- Highly Sensitive: limit of detection  $\sim\!2\mu M$  125 pmol
- Colorimetric measurement at 540-570 nm
- · Detection of NO2- in a linear and stable way

# LDH Cytotoxicity Assay Kit

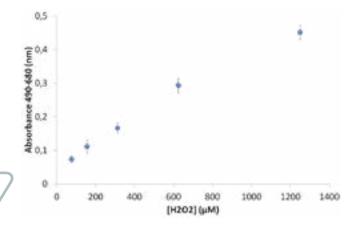
The **Nitric Oxide Detection Kit** is a colorimetric assay for the indirect determination of Nitric Oxide concentrations based on the enzymatic conversion of nitrate to nitrite by a nitrate reductase.

NO are important physiological messengers and effector molecules involved in many physiological processes such as vasodilation, inflammation, thrombosis or immunity (...). This assay is based on the reaction originally described by Griess to detect NO2– in a variety of biological fluids such as cell culture medium, plasma, serum, or urine.

### MAIN FEATURES

- No solubilisation process is required since this formazan does not require any solvation;
- Measurement can be performed directly in the tissue culture medium;
- Fast, Accurate, sensitive and adapted to high throughput screening

### LDH activity determined in HeLa cells



### RECOMMENDED APPLICATION

Measuring cell toxicity, late apoptosis or necrosis.

Cat. No.

Description

NOSO500

Nitric Oxide Detection Kit (25 mL reagent 1 (sulf.), 25 mL reagent 2 (NED)

1 mL Nitrite Standard (0.1 M))

LDH Cytotoxicity Assay Kit (12.5mL of 2X INT Buffer (A), 12.5mL of 2X Assa

LDH Cytotoxicity Assay Kit (12.5mL of 2X INT Buffer (A), 12.5mL of 2X Assay Buffer (B), 100µL of 1000X Buffer (C), 25mL Stop Solution, 1 mL Lysis Buffer (10X), 10 µL positive control)

500 assays

No. of assays

# TRANSFECTION TOOLS

Luciferi

DMNPE-caged Luciferir

G418 / X-Ga

GeneBlaster<sup>TI</sup>

pVectoz

HuoMas

R-Phycoethri

IgG FITC

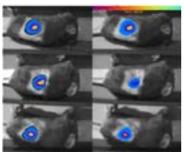
D-Luciferin K<sup>+</sup> and Na<sup>+</sup> salts are routinely used as Firefly's Luciferase substrate in *in vitro* & *in vivo* bioluminescent assays. The quality and purity of the D-Luciferin are essential to obtain good and reproducible results.

### **APPLICATIONS**

- Bioluminescent assays in living cells, tissues and animal models
- Luciferase reporter gene assays
- Whole animal imaging
- Appropriate read-out for transfection/ transduction with luciferase reporter gene and luciferase-fusion constructs
- ATP assays (luciferase catalyzes conversion of ATP into AMP) and immunoassays
- Pyrosequencing, luciferase fragment complementation for sequential gene experiments

Time course of D-Luciferin K+ luminescence signal

Comparison of OZ Biosciences' Luciferin sodium salt with competitors



la•Salt OZ Bio)

)-Luciferin Na\*Salt from C

D-Luciferin Na•Salt from I

70min

RECOMMENDED APPLICATION
In vivo & in vitro bioluminescent assays

### MAIN FEATURES

- High purity > 99.5%
- · Good solubility and great sensitivity
- Reliable in vivo detection
- Endotoxin-free (ideal for in vivo application)
- Quick and easy diffusion throughout the animal

▶ For bioluminescence please refer to Luciferase Assay Kit page 73

# **DMNPE-caged Luciferin**

The DMNPE-caged Luciferin is as a bioluminescent luciferase substrate used to measure intracellular functions. This D-luciferin ester analogue can readily cross cell membranes and can be used to supply a continuous source of active luciferin: once the caged luciferin is inside the cells, active luciferin can be released by the action of endogenous intracellular esterases or by a flash of UV light.

### MAIN FEATURES

- Cell permeable even at neutral pH
  -Efficient delivery of luciferin into living cells
  -Improved sensitivity analysis of *in vivo* luciferase assays
- Long-term measurement of luciferase activity
- Allows to follow changes in gene expression in live cells

### Chemical Structure

Cat. No. Product
LK10000 D-Luciferin potassium salt, 1 g
LN10000 D-Luciferin sodium salt, 1 g
LC10000 DMNPE-caged Luciferin 10 mg
LC25000 DMNPE-caged Luciferin 25 mg
LC50000 DMNPE-caged Luciferin 50 mg

The G418 Sulfate is an aminoglycoside antibiotic identical to gentamicin B1 produced by *Micromonospora rhodorangea*. It blocks polypeptide synthesis by inhibiting the elongation step in both prokaryotic and eukaryotic cells. It is used to select and maintain eukaryotic cells expressing the *neo* gene (neomycin). The quality and purity of the G-418 is essential to achieve good and consistent selection.

Molecular structure of G418 Sulfate

# RECOMMENDED APPLICATION Selection & maintenance of cells expressing the neo gene.

### MAIN FEATURES

• Alternative name: Geneticin

• Molecular formula:  $C_{20}H_{40}N_4O_{10}.2 H_2SO_4$ 

• Molecular weight: 692.71 g/mol

• **CAS number:** 108321-42-2

Molecular biology grade and premium pure

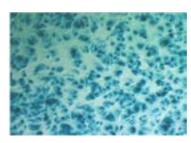
• Production & maintaining stably-transfected cells

# X-Gal substrate - Powder

The X-Gal substrate is metabolized by the  $\beta$ -galactosidase enzyme into an insoluble blue precipitate. It is ideal for staining transformed bacteria and LacZ transfected or infected cells, tissues and organisms. The quality and purity of the X-Gal substrate is essential to obtain high-quality and reliable results.

# RECOMMENDED APPLICATION Monitoring β-Galactosidase reporter gene activity

MCF-7 cells expressing β-Galactosidase enzyme



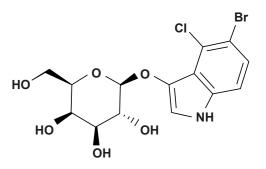
X-Gal is provided at 1g per vial. This product is also available in larger quantities (2g, 5g, 10g...).

Please contact us for a quote

### MAIN FEATURES

- High purity > 99% Molecular biology grade
- Good solubility and great sensitivity
- Perfect for cells, tissues & organisms staining

Chemical structure of X-Gal (5-bromo-4-chloro-3-in olyl-b-D-galactopyranoside)



Cat. No. XG31000

Product X-Gal subtrate, 1 g G-418 sulfate, 1 g To optimize your transfection experiments, OZ Biosciences has created GeneBlasters transfection

Boosters: an innovative and efficient solution to improve gene expression levels. GeneBlaster Kits

are a set of chemicals designed to get higher and longer transgene expression levels.

pVectOZ - Plasmid DNA vectors

pVectOZ are DNA vectors engineered in an optimized plasmid backbone. These plasmids encoding for the most popular reporter genes (CAT, GFP, LacZ, Luciferase, SEAP) are ideal for all transfections.

# APPLICATION

• These reagents offer solutions adapted to your scientific needs and cell sensibilities:

GeneBlaster Ruby: developed for adherent cells

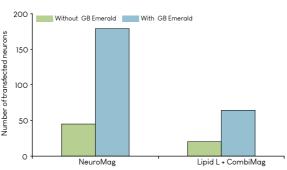
GeneBlaster Sapphire: developed for adherent cells complementing the Ruby

GeneBlaster Topaz: developed for suspension cells, especially hematopoietic

**GeneBlaster Emerald:** developed to improve transfection efficiency in neurons

RECOMMENDED APPLICATION
Enhancement and lengthening of transgene expression in adherent
& suspension cells

### Neuron transfection improvement with GeneBlaster Emerald

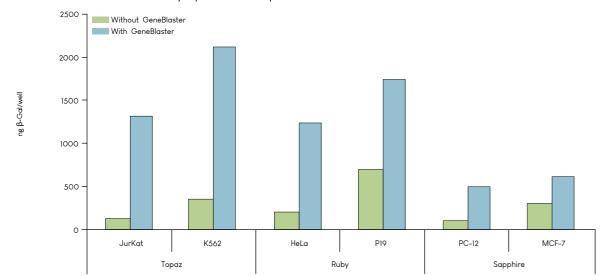


Primary hippocampal neurons were transfected with a pGFP plasmid DNA using NeuroMag (p.26) or a Lipid L + CombiMag (p.24) with and without Gene-Blaster Emerald. After 72H, the number of transfected neurons was estimated by fluorescence microscopy.

### MAIN FEATURES

- Higher gene expression in many cells
- Convenient for a large panel of adherent & suspension cells
- Prolong in vitro gene expression
- · Successful with all genetic vectors
- · Simple, rapid and easy-to-use
- Can be used with all commercially available transfection reagents

### Transfection efficiency improvement in the presence of GeneBlaster



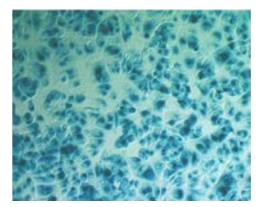
Cells seeded in 24-well plates were transfected with a pLacZ plasmid DNA using DreamFect Gold transfection reagent (cat #DG80500, see page 21) with and without GeneBlaster. β-galactosidase expression was monitored after 48H using OZ Biosciences' ONPG Assay Kit (p.69 #GO10001).

Cat. No.	Product	Kit contain	
GB20010	Selection Kit	1,5 mL vial of each reagent (x4)	
GB20011	GeneBlaster Ruby	3x1,5 mL, 450 assays	
GB20012	GeneBlaster Sapphire	3x1,5 mL, 450 assays	
GB20013	GeneBlaster Topaz	3x1,5 mL, 450 assays	
GB20014	GeneBlaster Emerald	3x1,5 mL, 225 assays	

All pVectOZ plasmids contain a modified human cytomegalovirus (CMV) promoter followed by specific intron, enhancer and terminator. The expression vectors are engineered in a optimized plasmid backbone to achieve the highest levels of transgene expression in mammalian cells and high copy number production in *Escherichia Coli*.

# RECOMMENDED APPLICATION Positive control & optimization of all transfection experiments

X-Gal expression in MCF-7 cells



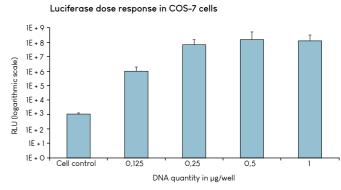
MCF-7 were transfected with 0.5 μg pVectOZ-LacZ using DreamFect Gold<sup>™</sup> transfection reagent. β-galactosidase activity was monitored 48H after transfection using the X-Gal Staining Kit (p.69 #GX10003).

### **FOCUS ON**

Two convenient packagings are available:

- 1. «Classical» 25 μg
- 2. 100  $\mu g$ : ready-to-use as controls in transfection

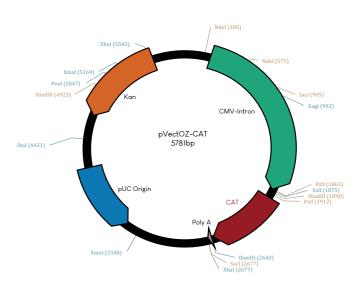
Save time & money by avoiding transformation, production & purification



COS-7 were transfected using several quantities of pVectOZ-LUC with DreamFect™ transfection reagent. Luciferase activity was measured 48H after transfection.

### MAIN FEATURES

- Highest levels of transgene expression in mammalian cells & tissues
- Suitable for all transfection applications: in vivo & in vitro
- High copy number production in Escherichia Coli
- Successful with all transfection reagents
- LPS-endotoxin free, supercoiled and highly purifiedtransfection grade approved



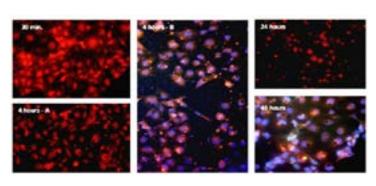
Cat. No.	Product	Contain
PL00010	pVectOZ CAT	25 µg of plasmid encoding for chloramphenicol acetyltransferase
PL00110	pVectOZ CAT	100 µg of plasmid encoding for chloramphenicol acetyltransferase
PL00020	pVectOZ GFP	25 µg of plasmid encoding for green fluorescent protein
PL00120	pVectOZ GFP	100 µg of plasmid encoding for green fluorescent protein
PL00030	pVectOZ LacZ	25 μg of plasmid encoding for β-galactosidase
PL00130	pVectOZ LacZ	100 μg of plasmid encoding for β-galactosidase
PL00040	pVectOZ Luc	25 μg of plasmid encoding for luciferase
PL00140	pVectOZ Luc	100 µg of plasmid encoding for luciferase
PL00050	pVectOZ SEAP	25 µg of plasmid encoding for secreted alkaline phosphatase
PL00150	pVectOZ SEAP	100 µg of plasmid encoding for secreted alkaline phosphatase

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# FluoMag - Improve your transgene expression

OZ Biosciences has created fluorescently-labeled magnetic nanoparticles for Magnetofection applications. FluoMag Transfection Reagents are as efficient as their non-labeled counterparts and allow you to visualize the nanoparticles in vitro during your experiments.

FluoMag-P corresponding to PolyMag (DNA transfection reagent, p.25), FluoMag-C corresponding to CombiMag (transfection reagent enhancer, p.26), FluoMag-N corresponding to NeuroMag (neuron transfection, p.28), FluoMag-S corresponding to SilenceMag (siRNA delivery, p.39), FluoMag-V corresponding to ViroMag (viral transduction, p.61).



### **APPLICATIONS**

- **Double labeling** and **co-localization** studies using GFP or FITC labeled nucleic acids
- Transfection mechanisms follow (interaction with cells, intracellular pathway ...)
- Fluorescent resonance energy transfer (FRET) assay
- Analyze the association of vectors with the magnetic nanoparticles

# R-Phycoerythrin

R-phycoerythrin (R-PE) is a naturally fluorescent protein from algae and is composed of a 240KD protein covalently linked to a number of fluorophores called phycobilins.

Its exceptional fluorescence characteristics make it a perfect positive control for intracellular protein delivery using OZ Biosciences Pro-DeliverIN reagent (cat. # PI11000).

R-phycoerythrin is provided at a 100µg/mL concentration in DPBS pH 7.4.

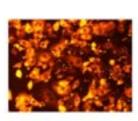
### MAIN FEATURES

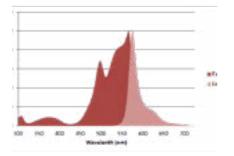
• Absorption maximum: 498 / 565 nm

• Emission maximum: 573 nm

• Extinction coefficient: 1.96 x 10<sup>6</sup> M-1 cm-1

• Quantum Yield: 0.84





# IgG-FITC

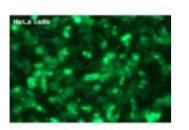
OZ Biosciences Immunoglobulin (IgG) from goat serum has been labelled with the fluorescein isothiocyanate dye to produce a green-emitting antibody with a high fluorophore-to-protein ratio.

The resulting antibody can be used as a positive control for intracellular antibody delivery with OZ Biosciences' Ab-DeliverIN reagent (cat. #AI21000).

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IgG-FITC is provided at a 100  $\mu g/mL$  concentration in sodium azide pH 7.4.





### MAIN FEATURES

 $\bullet$  Absorption maximum:  $492~\mathrm{nm}$ 

• Emission maximum: 520 nm

• Extinction coefficient: 75000 M-1cm-1

• Quantum vield: 0.79

Cat. No.	Product
FP10100	FluoMag-P 100µL
FC10100	FluoMag-C 100µL
FN10100	FluoMag-N 100 μL
FS10100	FluoMag -S 100µL
FV10100	FluoMag-V 100µL

Cat. No. PHY0100 IGG0100 Product R-Phycoerythrin 100µL IgG-FITC 100µL NOTES



Reagent Finder
Cell Transfection Database
FAQ & TIPS

# TECHNICAL RESSOURCES



TRANSFECTION TIPS

TECHNOLOGIES & APLLICATIONS

REAGENT FINDER / CITATION DATABASE

Protocols, MSDS, Results

DISTRIBUTORS





### TERMS OF SALE AND CONDITIONS

### GENERAL CONDITIONS OF SALE AND PAYMENT

OZ Biosciences, hereafter referred to as the "Seller".

### **GENERAL PRINCIPLES**

The present General Conditions apply to all sales placed with the Seller. The placing of an order implies the acceptance without reservation of these General Conditions. These Conditions may not be waived or modified by opposing terms appearing on any documents of the Buyer. No waiver by the Seller of strict compliance with any term of these Conditions shall constitute a waiver of any subsequent failure of the Buyer to comply strictly with each and every term and condition hereof. If any provision of these conditions of sale and payment shall be held invalid, the validity of the remaining provisions hereof shall not be affected thereby.

### USAGE

All the OZ Biosciences products are developed, designed, envisaged, and sold for the exclusive purpose of scientific research in laboratory. They are not in conformity with the requirements of the French, European and foreign pharmaceutical regulation. Consequently, they should not be employed for the human and veterinary diagnosis or be included/used in drug intended for the human use. The users are the only responsible for the uses, the experiments carried out and the handled products. The Buyer who wishes to use OZ Biosciences products for uses and/or applications not related to fundamental research must contact the direction of the company. For this purpose, OZ Biosciences reserves the right to accord or to refuse licenses for such uses.

### **ACCEPTANCE**

The orders are final only when the Buyer confirms them by writing. OZ Biosciences recommends using the numbers and designations of the catalogue or the concerned offer. In the case of unclear wording, if the Salesman must make a choice itself, it declines his responsibility; the expenses of return for nonconformity, which will result from this, will be the Buyer responsibility.

### **PRICES**

Our prices are net, quoted ex-works, taxes excluded, in euros or USD and based on the communicated prices to the customer. Our prices exclude shipment. Prices quoted in any documentation of the seller are without undertaking as regards the duration of validity and are subject to change between two orders. OZ Biosciences reserves the right to modify, without notice, its products price. Prices invoiced shall be those of the price list in force on the date of order.

### SHIPMENTS

The Seller shall arrange for the packaging in a manner suitable under normal transport conditions to prevent damage to or deterioration of the goods taking into account their destination. Deliveries are made ex-works. The shipment costs are the responsibility of the customer. Should the Seller accept to arrange for the transportation, according to the Buyer's instructions, any and all forwarding charges shall be invoiced in addition to the Buyer. The delivery is carried out either by the direct handling-over of the goods to the customer, or by notice of delivery, or by delivery with a transporter or a shipper. Whatever the conditions of expeditions are, our goods travel to the risks and dangers of the recipient and without insurance. In case of damage and being lost in the course of shipment, the Buyer will have to notify the shipper the damage or lacks noted within the legal times and to inform the Salesman of this notification within the same times. The Salesman declines any responsibility in the event of nonobservance for these formalities. Our delivery periods are indicative. No allowance for delay of delivery could be claimed. The delivery can be made only if the Buyer is up to date of his obligations towards OZ Biosciences.

### RETRUNS

No return will be accepted without prior agreement and written from our Sales management, which will specify the methods of return. In this case, the articles will be returned, in their packing of origin, in paid port, to the address which will be communicated to the Buyer. OZ Biosciences reserves the right to send back, in paid port, all goods received without this agreement.

### FORCE MAJEURE

The Seller shall be entitled to cancel the whole or any order the fulfillment of which has been suspended or is no longer possible due to causes of any kind or extent beyond the Seller's control or of force majeure, including but not limited to war, partial or total strikes, breakdown of transportation, shortage of raw material, fires, fioods, tooling accidents or any other circumstances impeding the activity of the Seller's works.

### **CANCELATION OF ORDERS**

Any order is binding upon the Buyer and irrevocable when accepted by the Seller. No order may be cancelled by the Buyer, except with the Seller's prior written consent, in which case the Seller reserves the right to claim as indemnity the value of manufactured goods or of the work in progress.

### **DESCRIPTION - CHARGES IN PRODUCT**

Descriptions and specifications appearing in the Seller's documentation are given as a guide only. The Seller reserves the right at any time and from time to time to make changes to the products in such a manner, as it may consider advisable particularly to have them conform to technical developments, but the Seller shall not be held to make such charges to its products previously delivered or the delivery of which is in progress. Any and all drawings, descriptions, specifications, proposals, price-lists and more generally any documents issued by the Seller are the Seller's proprietary information and cannot be used, reproduced or disclosed to third parties, except with the Seller's prior express agreement.

### LACK OF CONFORMITY - CLAIMS

Any claim relating to lack of conformity must be notified by registered letter together with a bill of receipt and requested within a 48-hour delay from the receipt of goods. Any use of the goods shall be considered as a waiver by the purchaser of the right to claim for lack of conformity.

### WADDANTY

Warranty of the reagents /products occurs only if packing is stored under good conservation conditions. The materials and new equipment sold by OZ Biosciences are guaranteed against all manufacture defects for one year as from the delivery. This guarantee is applicable exclusively in the event of defect coming from design or hidden deficiency.

### **PAYMENT CONDITIONS**

All payments shall be due 30 days from the date of invoice, net and without credit, even in the case of cash payment. The payment of any partial delivery becomes eligible at the due date mentioned on the corresponding invoice, and not at the time of the balance dues. OZ Biosciences reserves the right to claim an installment before the order execution. Any deterioration of the Buyer credit could justify the demand of guarantees or require cash payment, before the execution of the received orders. In accordance with the legal provisions, if the payment is not made at the date stated on the invoice, interest on the delay of payment will be payable based upon three time the current bank rate from the day following the date upon which the payment was due and must be paid in addition to the amount stated on the invoice and the Sellers reserves the right to suspend the fulfillment of the any possible pending delivery. If the Buyer has past due balances or if its financial standing worsens seriously, the Seller reserves the right to require cash payment before execution of any further delivery, notwithstanding the usual conditions of payment. No compensation for any possible sums in litigation or any blocking of the payment of the invoices will be accepted. In the event of possible litigation all the expenses shall be borne buy the Buyer..

### **RETENTION OF TITLE - CANCELLATION**

All goods delivered remain the seller's property until payment in full of their price. The transfer of title shall arise solely upon actual collection of price. The purchase of OZ Biosciences products grants the purchaser a non-transferable, non-exclusive license to use the products and/or its separate and included components. These products are intended for in-house research only by the buyer. In addition, research only use means that the products and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences. Title on goods being retained by the Seller until full payment of their price, it is expressly understood that the purchaser is not authorized to sell, pledge or in anyway dispose of the goods before such payment. Notwithstanding the retention of title, the purchaser shall bear any and all risks the good could undergo or cause as from the delivery of the goods. Should payment not have been made on the due date for the total or partial amount of the invoice, the Seller shall have the right to cancel any and all sales delivered but not paid for and to take back the goods, by notifying the purchaser of its intent by registered mail together with a bill of receipt and requested eight days before the taking back. The costs of return of the goods shall be due by the purchaser in default, together with any depreciation of the goods. The Seller as compensation shall retain installments previously paid.

### JURISDICTION

The players will seek, before any contentious action, a friendly agreement. The Tribunal of Commerce of Marseille (France) shall be the only competent party to settle any dispute, in the event of litigation, resulting from an order, unless OZ Biosciences prefer to seize any other competent court of jurisdiction. This condition may not be waived or modified by opposing terms appearing on any documents of the Buyer, even in the event of summary procedure, of incidental request or plurality of defendants or in calls of guarantee. No waiver by the Seller of strict compliance with any term of these Conditions shall constitute a waiver of any subsequent failure of the Buyer to comply strictly with each and every term and condition hereof.