Magnetofectamine™ - *Results*

The two most powerful transfection technologies in one kit!

Magnetofectamine™ is the association of **Lipofectamine™ 2000** the most renowned transfection reagent from Invitrogen™ with the unique Magnetofection™ based reagent **CombiMag**, from OZ Biosciences.
The alliance of **Lipofectamine™ 2000** and **CombiMag** reagent leads to increased transfection efficiency, minimized toxicity and enhanced gene expression. It is specially suited for hard-to-transfect and primary cells.

Magnetofection™ is a creative, simple and highly efficient method to transfect cells *in vitro* and *in vivo* *. This magnetic assisted transfection technique is based on the association of magnetic nanoparticles with nucleic acids (alone or pre-complexed with a transfection reagent) or with viruses to which a magnetic field is exerted. The magnetic force drives the gene vector towards the target cells, allows a rapid concentration of the vector dose onto the cell and triggers delivery via endocytosis. Consequently, high transfection efficiencies can be achieved with less nucleic acid amount.

Lipofectamine™ 2000 transfection reagent is a proprietary formulation of Invitrogen™ for transfecting nucleic acids (DNA and siRNA) into eukaryotic cells.
Lipofectamine™ and Invitrogen™ are Trademarks owned by Life Technologies Corporation. Lipofectamine™ 2000 is manufactured by Life Technologies Corporation for OZ Biosciences and provided under license from Life Technologies Corporation.

Magnetofectamine™ principal advantages:

1. Improved transfection efficiency
2. Enhanced Lipofectamine™ 2000 efficiency
3. Ideal for hard-to-transfect and primary cells
4. Less nucleic acids used - minimized toxicity
5. No need to change your standard Lipofectamine™ 2000 protocol
6. Compatible with any types of nucleic acid and any culture medium
7. Simple, ready-to-use & rapid

Applications

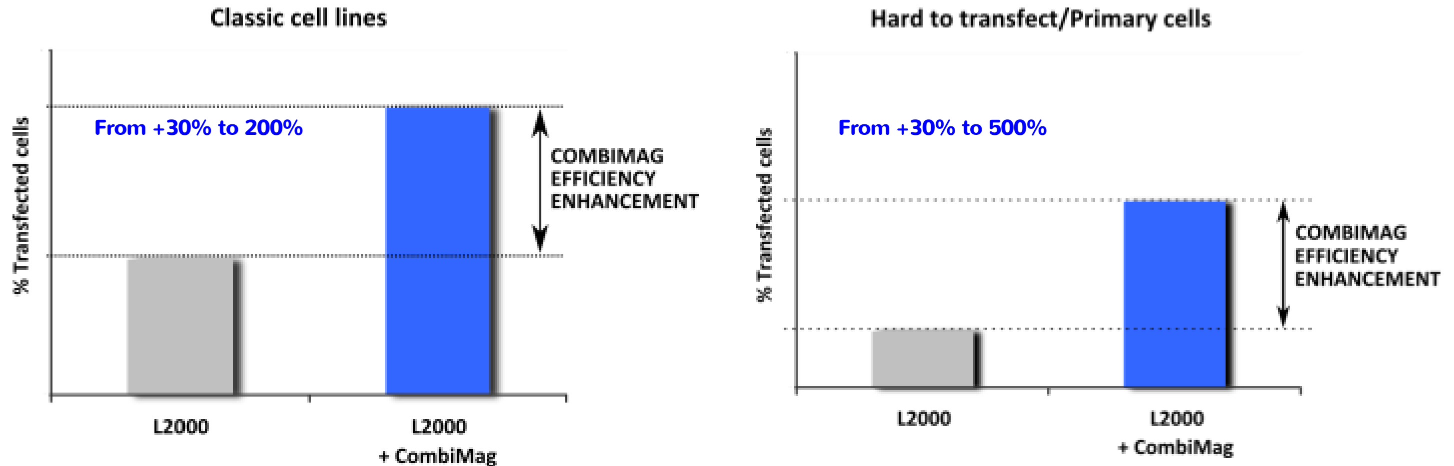
Magnetofectamine™ Kit associates Lipofectamine™ 2000 transfection reagent and CombiMag, the magnetic nanoparticles designed to be combined with any transfection reagents and to boost their efficiencies. The combination of the two technologies enables using smaller amounts of nucleic acid and increasing the overall efficiency of your transfection. For further information concerning the Magnetofection™ technology, see our website: www.ozbiosciences.com.

Magnetofectamine™ Kit can be used with various types of nucleic acids (plasmid DNA, siRNA, oligonucleotides, linearized DNA, double stranded RNA, mRNA, shRNA ...).
It is suitable for numerous cell types and is particularly useful for very difficult-to-transfect cells & primary cells. An updated list of successfully transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.
Magnetofectamine™ Kit is serum compatible and can be used for transient and stable transfection.
These products are very stable, ready-to-use and intended for research purpose only (not intended for diagnostic use or any animal and human therapeutic).

** Plank C, Zelphati O, and Mykhaylyk O. Magnetically enhanced nucleic acid delivery. Ten years of magnetofection-Progress and prospects. Adv Drug Deliv Rev. 2011; 63 (14-15):1300-31.*

CombiMag Enhances Lipofectamine™ 2000 Efficiency

CombiMag enhances the transfection efficiency of Lipofectamine™ 2000.

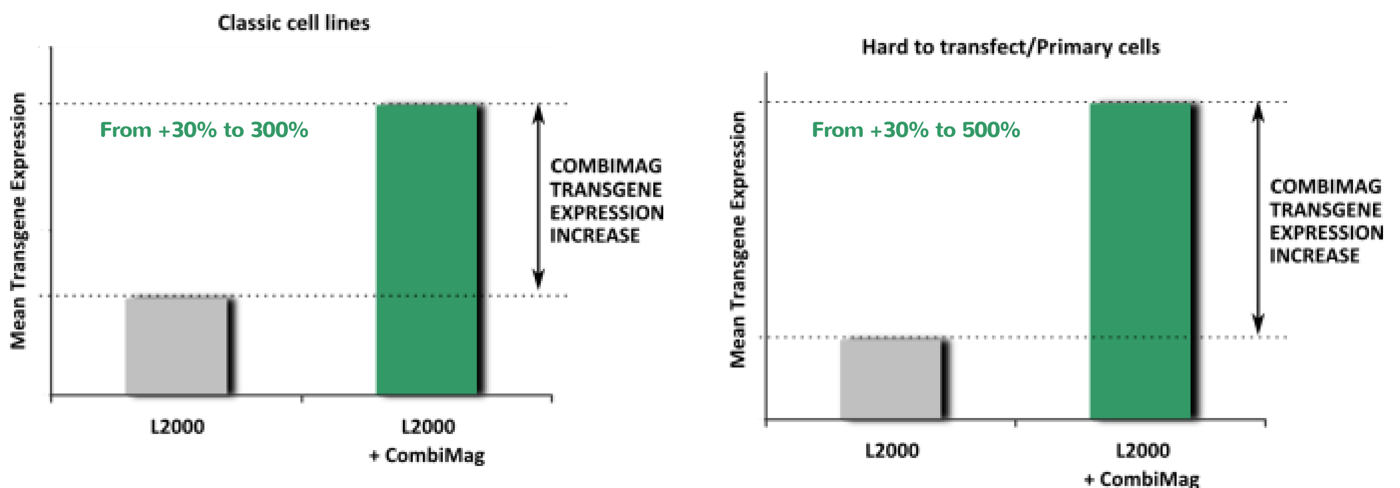


Average percentages of cells transfected with Lipofectamine™ 2000 reagent or with Magnetofectamine™ (Lipofectamine™ 2000 and CombiMag).

Left: Graph represents a graphical view of average percentages of common cell lines (HEK-293T, HeLa, COS-7, NIH-3T3, BEAS-2B, CHO-K1, MCF-7, Vero) transfected with Lipofectamine™ 2000 (grey bar) or with Magnetofectamine™ (Lipofectamine™ 2000 and CombiMag; blue bar).

Right: . Graphical representation of the mean percentage of hard-to-transfect/primary cells such as HMEC-1, RAW 264.7, MDCK, HUVEC, SH-SY5Y, mesenchymal stem cells, neurons, endothelial and neural stem cells transfected either with Lipofectamine™ 2000 alone (grey bar) or with Magnetofectamine™ (Lipofectamine™ 2000 and CombiMag; blue bar).

CombiMag increases the transgene expression level induced by Lipofectamine™ 2000.



Average levels of transgene expression by cells transfected with Lipofectamine™ 2000 reagent or with Magnetofectamine™ (Lipofectamine™ 2000 and CombiMag).

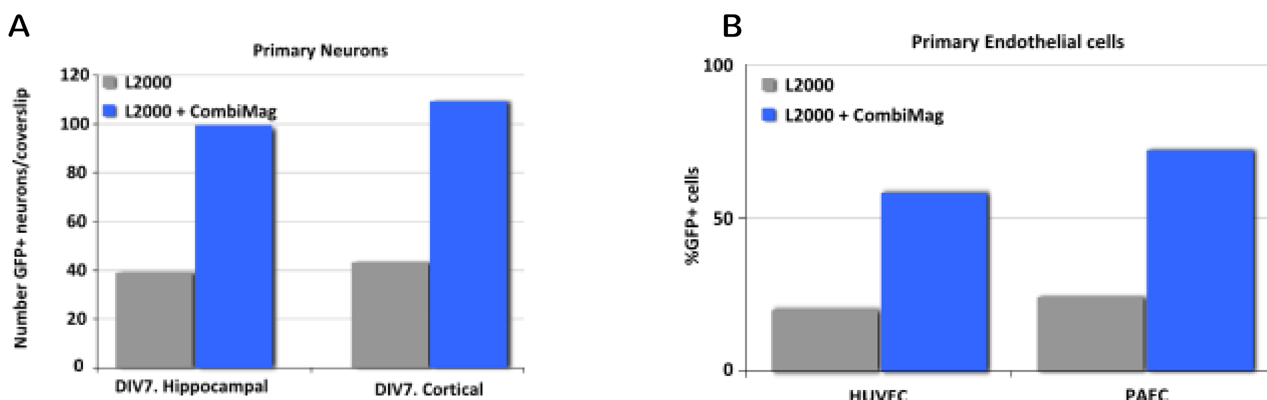
Left: Graph represents a graphical view of average transgene expression by classical cell lines (HEK-293T, HeLa, COS-7, NIH-3T3, BEAS-2B, CHO-K1, MCF-7, Vero) transfected with Lipofectamine™ 2000 (grey bar) or with Magnetofectamine™ (Lipofectamine™ 2000 and CombiMag; green bar).

Right:. Results show a graphical representation of the mean transgene expression level by hard-to-transfect/primary cells such as HMEC-1, RAW 264.7, MDCK, HUVEC, SH-SY5Y, mesenchymal stem cells, neurons, endothelial and neural stem cells transfected either with Lipofectamine™ 2000 alone (grey bar) or with Magnetofectamine™ (Lipofectamine™ 2000 and CombiMag; green bar).

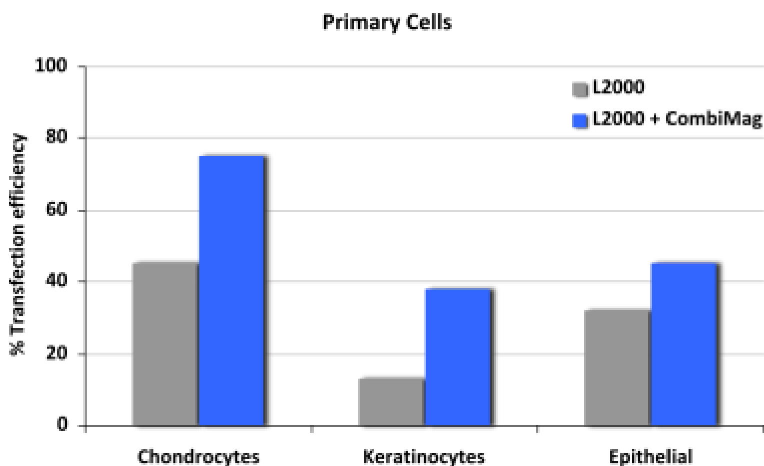
Magnetofectamine™ Efficiency for Primary Cells

DNA Transfection

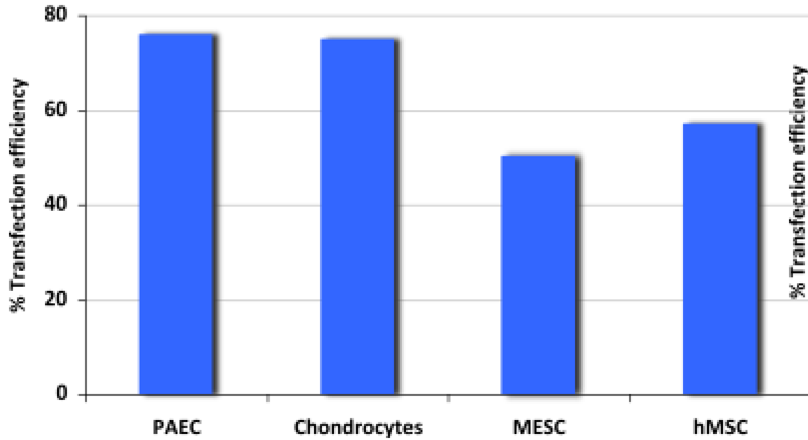
Magnetofectamine™ kit enhances DNA transfection efficiency in a variety of primary cells. The list of primary cells successfully transfected with Magnetofectamine™ as well as the list of published papers are available on our website: www.ozbiosciences.com. For example: ***neurons, endothelial, epithelial, fibroblasts, pituitary, stroma mesenchymal, myocytes, Parietal, cardiomyocytes, chondrocytes, hepatocytes, stem, keratinocytes, smooth muscle cells***...

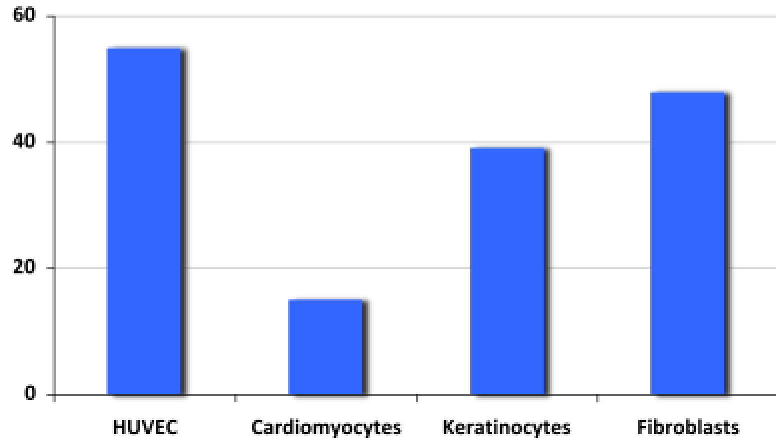


(A) Primary hippocampal and cortical neurons and (B) primary endothelial cells were transfected with Lipofectamine™ 2000 or Magnetofectamine™ (Lipofectamine™ 2000 + CombiMag). Results showed that CombiMag enhanced Lipofectamine™ 2000 transfection efficiency on all primary cell types. *Figure (B) is adapted from Basile et al.* 2005. Mol Cell Biol. 25:6889-6898 (1).

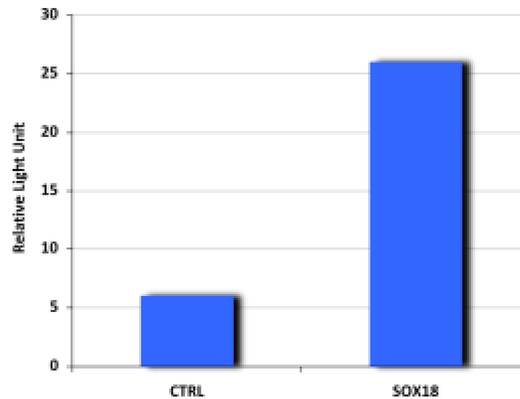


Various Primary cells were transfected with Lipofectamine™ 2000 or Magnetofectamine™. Results showed that CombiMag enhanced Lipofectamine™ 2000 transfection efficiency for all cells.



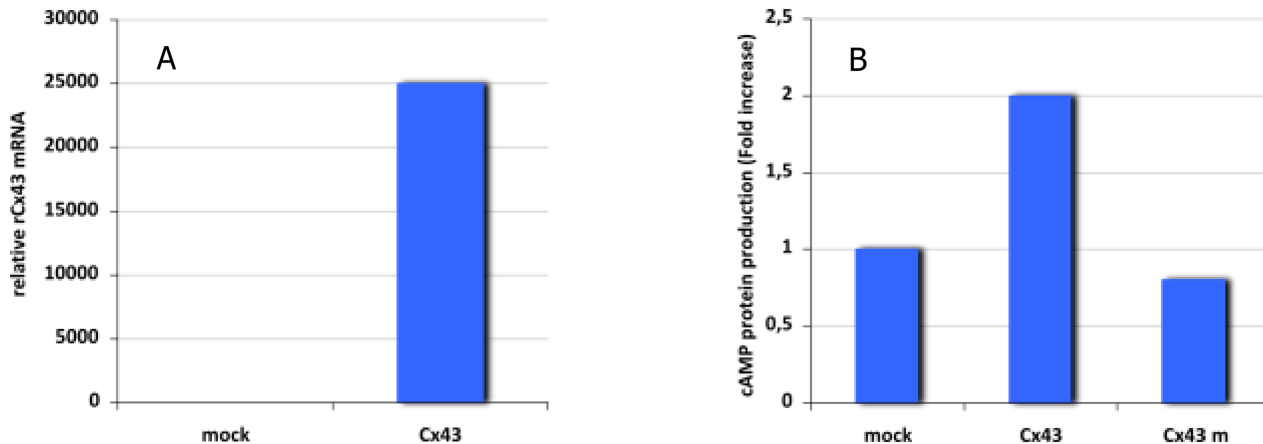


- Primary mouse mesenteric lymph node endothelial cells



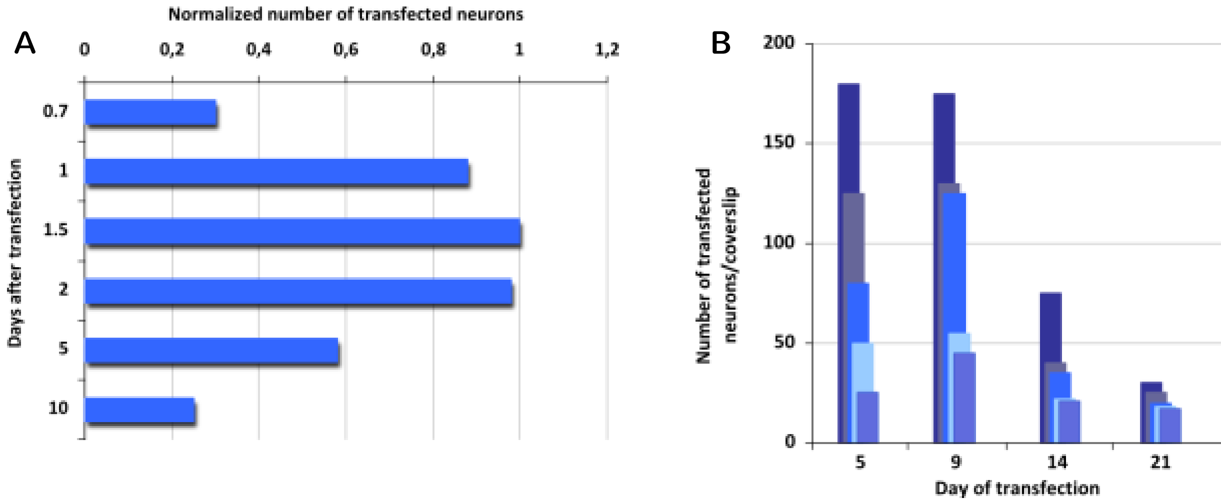
François *et al.* co-transfected a fusion plasmid encoding for luciferase under prox1 promoter and a plasmid encoding sox18, the promoter trans-activator into primary mouse mesenteric lymph node endothelial cells (mlEnd) using Magnetofectamine™. Results demonstrated that overexpression of Sox18 led to a significant increase in luciferase activity. *Figure adapted from François et al.* 2008. Nature. 456:643-647*(2).*

- Primary human retinal epithelial cells

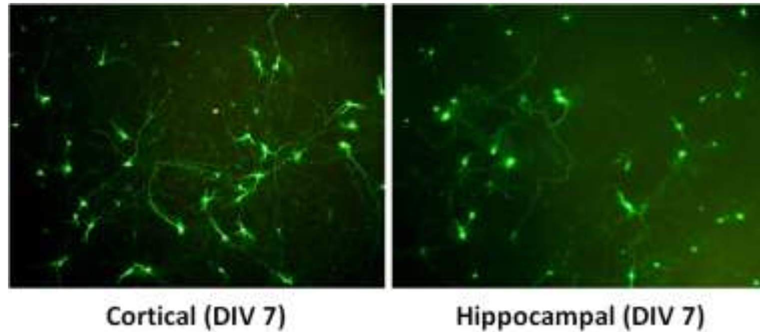


In human retinal pigment epithelial cells Kojima *et al.* transfected a plasmid encoding for Cx43, a major gap junction protein. 10^5 cells/well in a 6-well plate were cultivated in DMEM and transfected with Magnetofectamine™. (A) Relative amount of Cx43 mRNA was measured in cells transfected with the plasmid encoding for Cx43 or with a mock plasmid. (B) Production of cAMP related to Cx43 expression was measured in cells transfected with mock, Cx43 or mutant Cx43 plasmid DNA. *Figure adapted from Kojima et al. 2008. Biochem. Biophys. Res. Commun. 366:532-538 (3)*

- Primary neurons



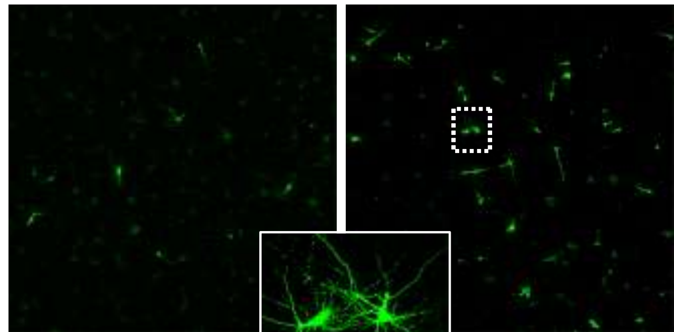
Buerli T *et al.*, in Nature Protocol, described step by step the procedure to reach the highest transfection efficiency in primary neurons with Magnetofectamine™. This publication is now a reference article for transfecting long term neuronal cultures. (A) Neurons were transfected at DIV 9 and neurons expressing eGFP protein were counted and expressed as a relative number per 14mm coverslip at different time points. (B) Neurons were transfected at different DIV and neurons expressing eGFP were quantified after 36h transfection with Magnetofectamine™. *Figure adapted from Buerli et al. 2007. Nat Protoc. 2:3090-3101 (4).*

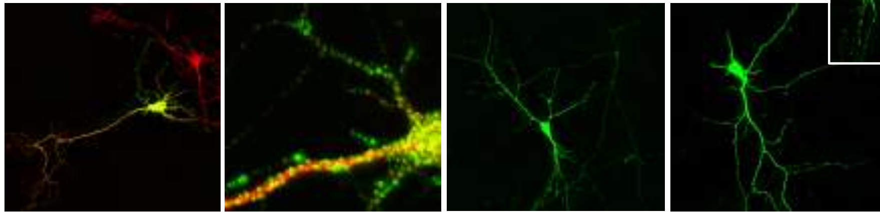


Rat primary cortical and hippocampal neurons (DIV 7) were transfected using 1 g / well of pVectOZ-GFP plasmid and Magnetofectamine™ (Lipofectamine™ 2000 + CombiMag) at a 1:1 ratio. Transfection efficiency was assessed by fluorescence microscopy 48 h post-transfection. Results showed that CombiMag enhanced the number of GFP+ neurons when added to Lipofectamine™ 2000.

Primary Rat Hippocampal Neurons
Transfected 14 d.i.v, with Lipofectamine™ 2000 +CombiMag
Reporter Gene: GFP
Culture dish: 35 mm
DNA: 1µg / well

We are thankful to Dr Igor MEDINA (INSERM U29, Marseille) for kindly providing these data.





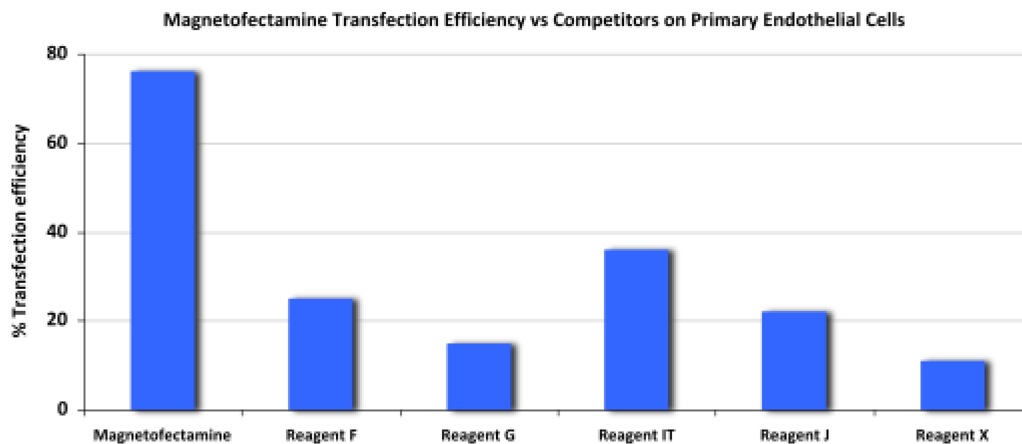
"CombiMag give us reliable and improved transfection efficiency up to 300% enhancement" Dr.I.Medina

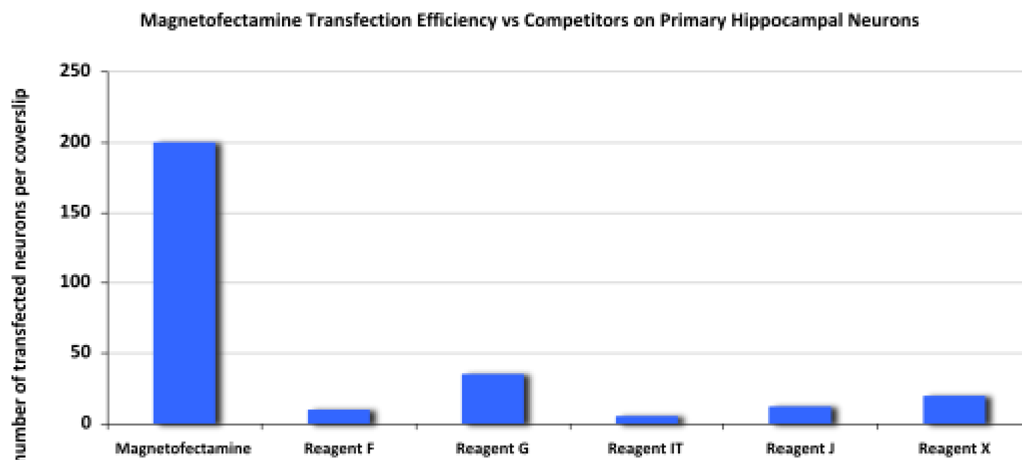
Primary neurons were transfected using 1 µg DNA complexed to Lipofectamine™ 2000 and to 1 µL CombiMag at 14 DIV. *Results kindly provided by Dr. I. Medina (INMED).*

Numerous publications have demonstrated the Magnetofectamine transfection efficiency in primary hippocampal (4-15), cortical (4, 16-19), neocortical (20), and embryonic DRG (16) neurons.

Altogether, the results confirm the efficiency of Magnetofectamine to transfect DNA into primary neurons or primary/hard-to-transfect cells.

DNA Transfection Efficiency vs Competitors

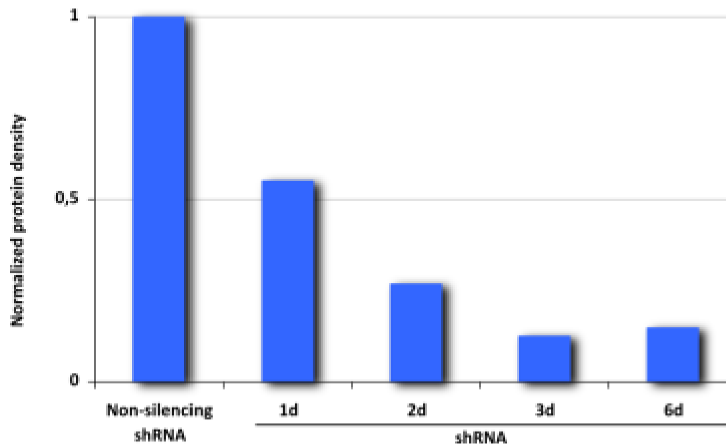




Gene Silencing

- shRNA

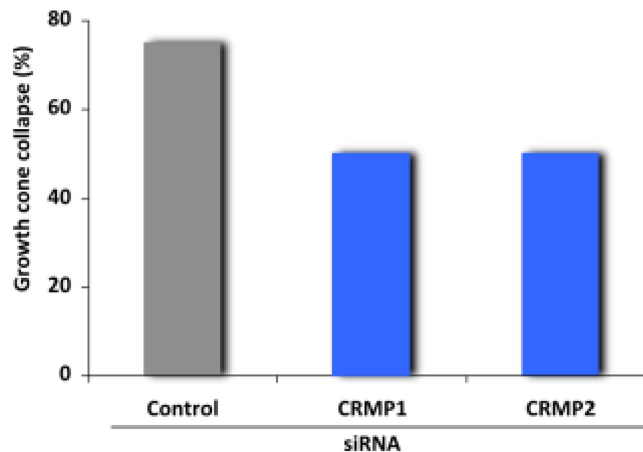
Magnetofectamine™ is suitable for gene knockdown using vectors expressing RNAi-inducing shRNAs.



A shRNA encoding vector was transfected in neuronal cultures using Magnetofectamine™. Three days after Magnetofection and beyond (up to 6 d), clusters of protein of interest were not detectable anymore in all transfected neurons. *Figure adapted from Buerli et al. 2007. Nat Protoc. 2:3090-3101 (4).*

- siRNA

Magnetofectamine™ is also suitable for gene silencing using siRNA in co-transfection experiments.



Magnetofectamine™ was used in primary cultures of rat embryonic dorsal root ganglion neurons (E14) to increase efficiency for gene silencing. siRNA were co-transfected with a plasmid encoding for a gene of interest and 24 hours later, the rates of growth cone collapse of DRG neurones transfected with siRNA were compared. Results showed the capacity of Magnetofectamine™ to target gene of interest without impairing the cell morphology and behaviour. *Figure adapted from Uchida et al. 2005. Genes Cells. 10:165-179 (16).*

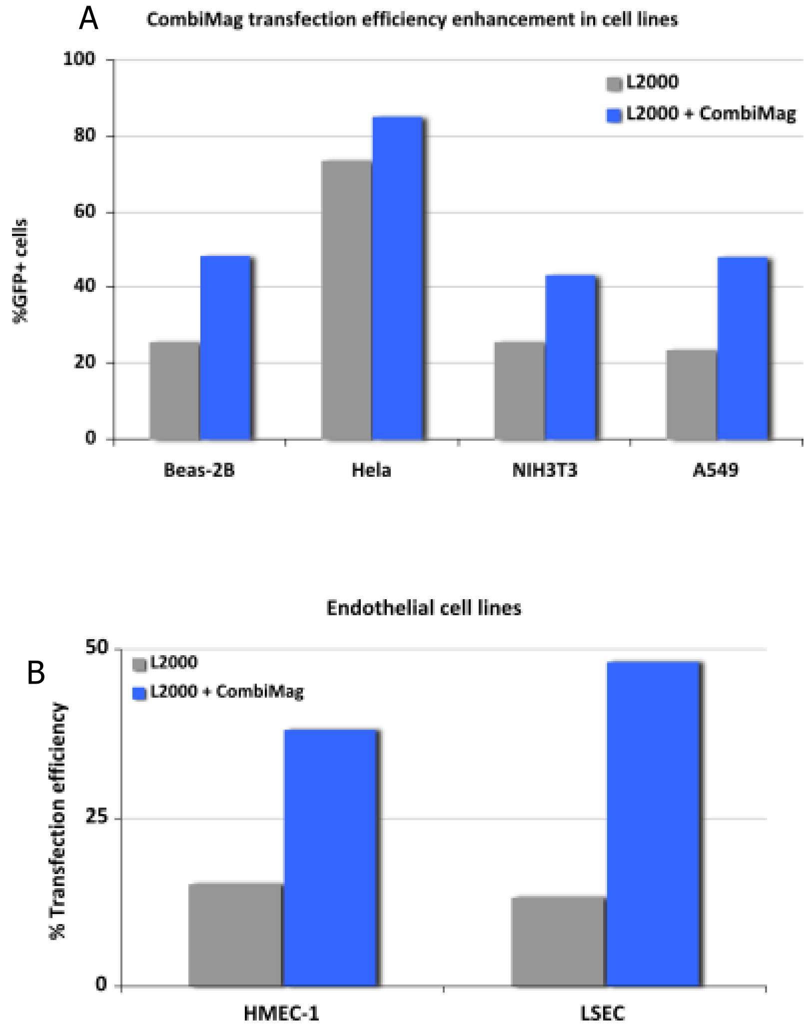
Altogether, the results confirm the efficiency of Magnetofectamine for gene silencing into primary neurons or primary/hard-to-transfect cells.

Magnetofectamine™ Efficiency for Cell Lines

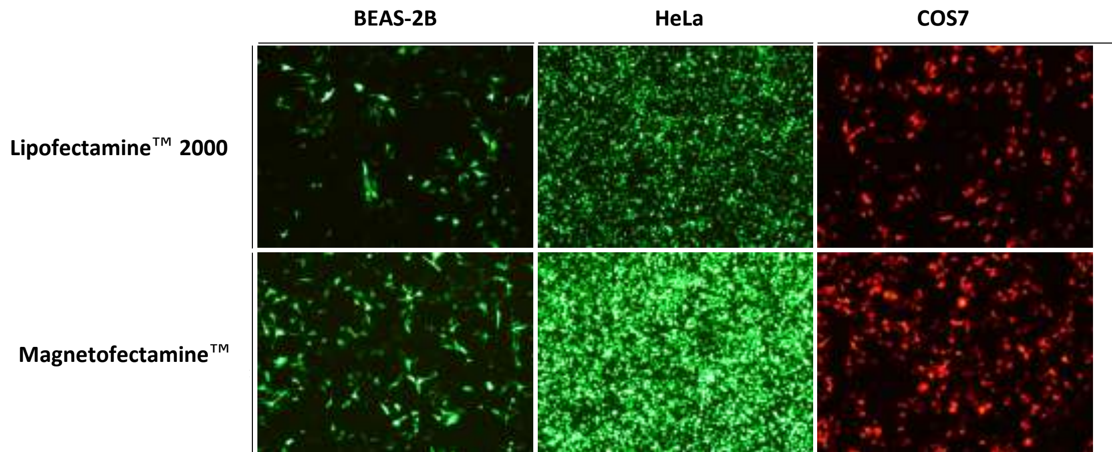
DNA Transfection

Even if the most striking efficiency of CombiMag is noticed in primary cells, its efficiency to improve Lipofectamine™ 2000 transfection capacity was also demonstrated in classic cell lines such as A549 (21), CaCo-2 (22), CHO-K1 (23, 24), H295R (25-27), HEK-293 (23), HeLa (24), Huh-7 (22), MCF-7 (28), MN9D (29, 30), N2A (17), SH-SY5Y (6, 24), TIG3 (31), Neuro-2A (32).

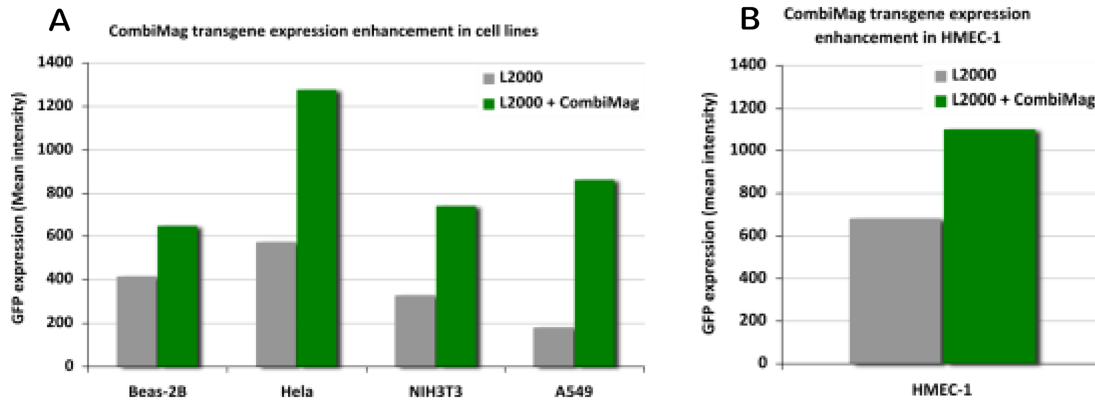
- Cell lines



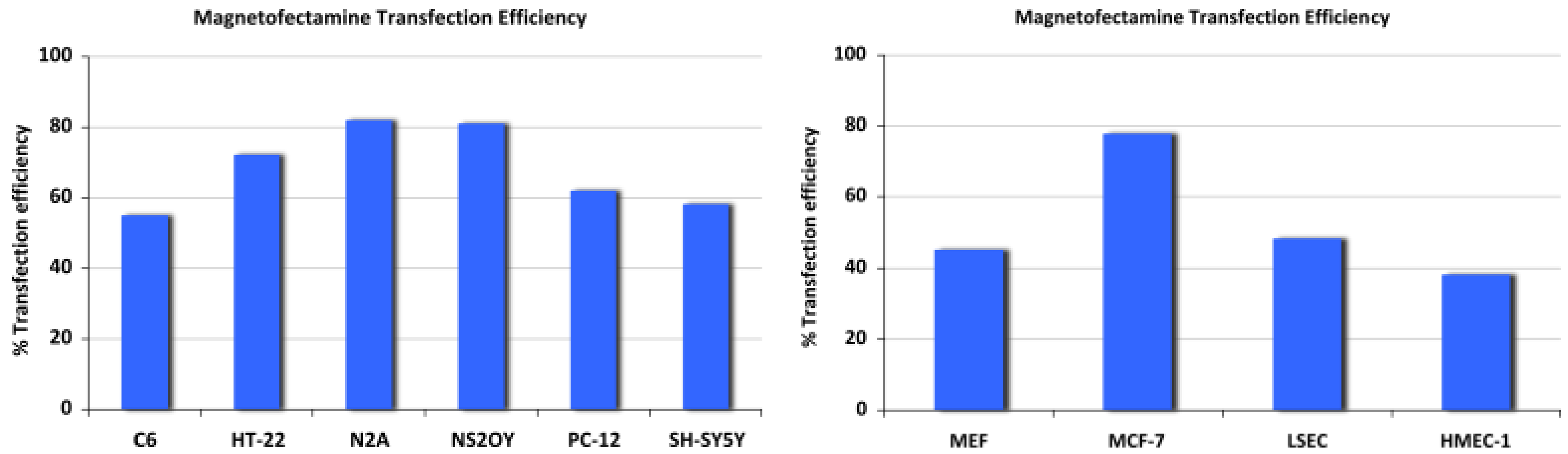
(A) Classical cell lines and (B) endothelial cell lines were transfected using 0.25 µg and 0.5 µg pVectOZ-GFP plasmid respectively complexed with Lipofectamine™ 2000 (grey bars) or with Lipofectamine™ 2000 and CombiMag at a DNA/CombiMag ratio of 1:1 (blue bars). Results were analysed by flow cytometry 24 h after transfection.



BEAS-2B, HeLa and COS7 cells were transfected using 0.25 µg pVectOZ-GFP plasmid or DsRed plasmid complexed with Lipofectamine™ 2000 or with Magnetofectamine™ kit using a DNA/CombiMag ratio of 1:1. Images were acquired 24h after transfection.

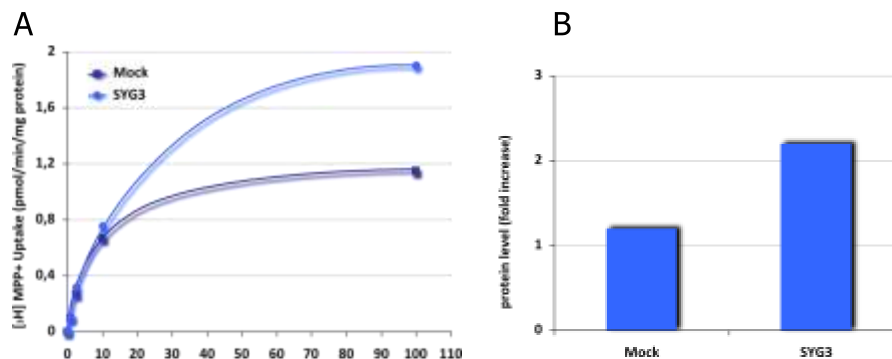


(A) Classical cell lines and (B) HMEC-1 were transfected using 0.25 µg and 0.5µg pVectOZ-GFP plasmid respectively complexed with Lipofectamine™ 2000 (grey bars) or with Lipofectamine™ 2000 and CombiMag at a DNA/CombiMag ratio of 1:1 (green bars). Results were analysed by cytofluorometry 24 h after transfection.



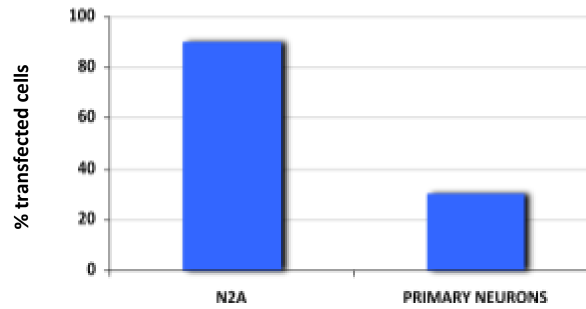
Various cell lines were transfected with Magnetofectamine™ kit in 24-well plate and efficiency was monitored 48h ours post-transfection by flow cytometry.

- Mouse dopaminergic neuronal cell line (MN9D cells)



Egana *et al.* used Magnetofectamine™ to generate stably transfected DAT-expressing clones (MN9D-DAT cells). The authors further transfected these clones with synaptogyrin-3 and measured the dopamine transporter (DAT) activity. (A) MN9D-DAT cells transfected with synaptogyrin-3 vector showed an increased in uptake activity compared with MN9D-DAT mock-transfected cells. (B) Overexpression of synaptogyrin-3 results in a twofold increase of protein levels. *Figure adapted from Egana et al. 2009. J Neurosci 29:4592-4604 (29).*

- Neuroblastoma cell line (N2A cells)

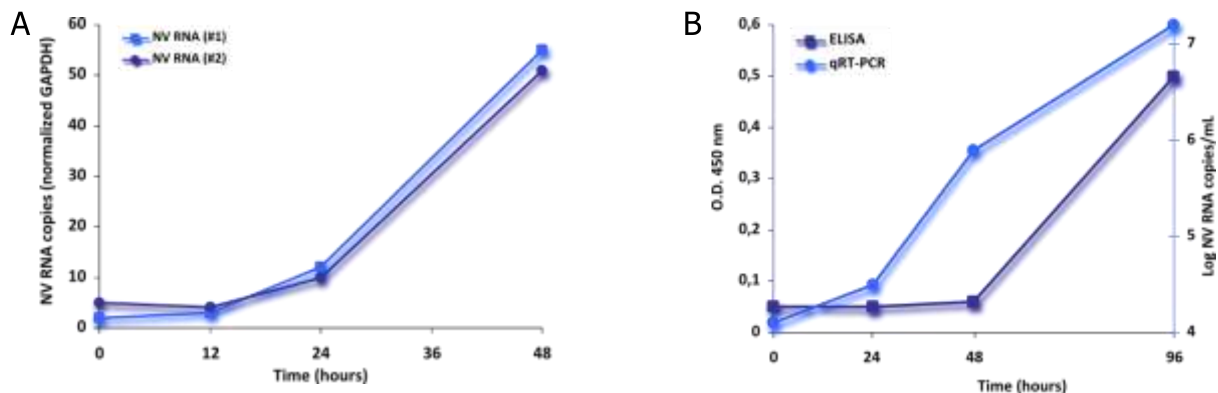


Tan Z. *et al.* transfected both N2A cell line and rat primary neurons with Magnetofectamine™. Cells were co-transfected with two plasmids encoding for DsRed2 and UbB+1 genes. Co-transfection results showed that almost 90% N2A and 30 % primary neurons were transfected. *Figure adapted from Tan et al. 2007. Cell Death Differ 14:1721-1732 (17).*

Altogether, the results confirm the efficiency of Magnetofectamine for DNA transfection into a variety of cell lines.

RNA Delivery

- Human hepatoma cell lines



Guix S. *et al.* used Magnetofectamine™ to demonstrate that transfection of Norwalk Virus (NV) RNA into human hepatoma leaded to viral replication, expression of viral antigen and release of viral particles into the medium. (A) Levels of two types of NV RNA into transfected cells at different time points. (B) Detection of NV particles in the supernatant of transfected cells measured by ELISA and qRT-PCR. *Figure adapted from Guix et al. 2007. J Virol 81:12238-12248 (22).*

The results demonstrate the efficiency of Magnetofectamine for RNA delivery into cell lines.

Ex-vivo **and** ***in-vivo Magnetofectamine™*** **efficiency**

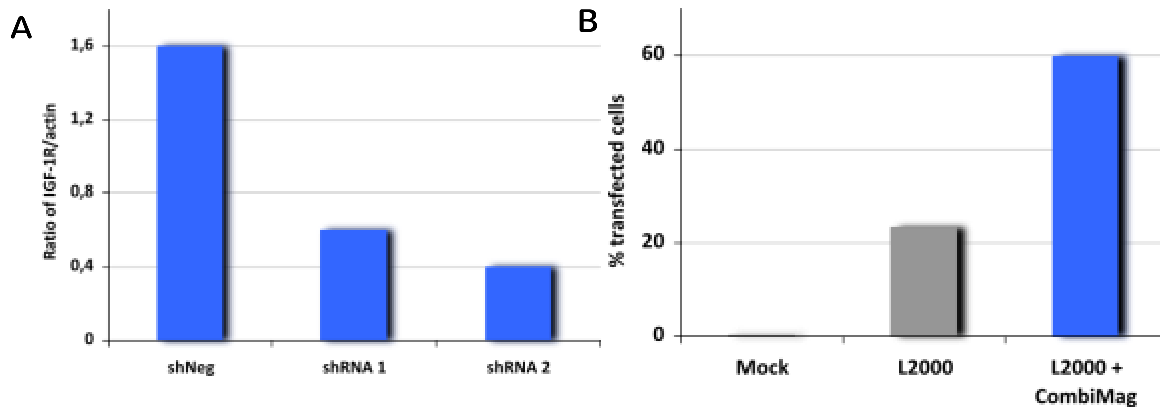
- Ex Vivo

Svingen *et al.*, in 2009 have used Magnetofectamine™ to transfect genital ridges *ex-vivo*. After tissues dissection, transfections were performed using 2.5 µL Lipofectamine™ 2000 and 3.5 µl CombiMag per µg DNA. Magnetic complexes were injected using glass capillary into genital ridges before placing them onto a magnetic field for 1 hour. Using this method, the authors showed a localised effect of their gene of interest stating that "magnetofection may provide a rapid indicator of gene function during organ development, a system that is likely to be broadly applicable in developmental biology". *Summary of Svingen et al. 2009. Dev Dyn 238:956-964. (33)*

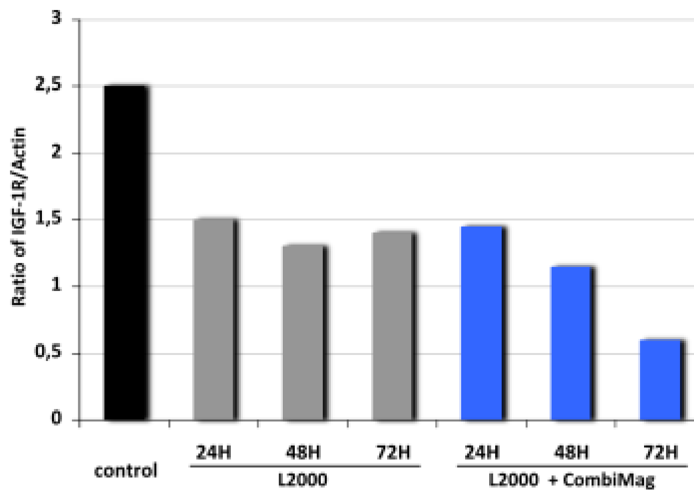
The results show the efficiency of Magnetofectamine for ***ex-vivo*** **DNA delivery**

- In Vivo

Recently, Wang *et al*. successfully associated CombiMag and Lipofectamine™ 2000 to transfect targeted areas *in vivo*.



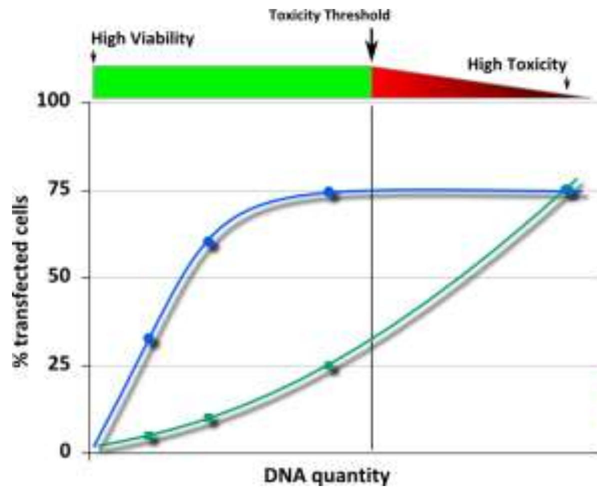
First, the authors confirmed the efficiency of the Magnetofectamine™ kit *in vitro*: (A) IGF-1R gene expression in human A549 cells after transfection using Magnetofectamine kit with two plasmids encoding for shRNA directed against gene of interest. (B) Lipofection (L2000) and Magnetofectamine were used with a GFP encoding plasmid in A549 cells.

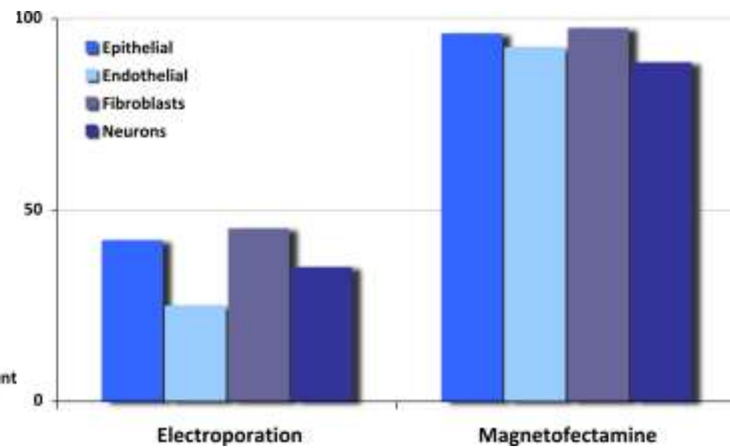


Then the authors silenced IGF-1R protein expression into A549-induced tumors *in vivo*. After tumour induction by A549 cells subcutaneous injection, PBS (control), 50µg plasmid encoding shRNA/125 µL lipofectamine™ 2000 (L2000) and 50 µL shRNA encoding plasmid/50 µg CombiMag/125 µL lipofectamine™ 2000 (L2000+CombiMag) were injected into tail vein. Prior to Magnetofection, a magnet was positioned onto the tumour surface for 15 min. Tumours were removed at 24, 48 and 72H post transfection and IGF-1R protein production was analysed. *Figures adapted from Wang et al. 2011. Biochem Biophys Res Commun 410:537-542 (21).*

The results show the efficiency of Magnetofectamine™ for *in-vivo* DNA delivery

Viability and Efficiency

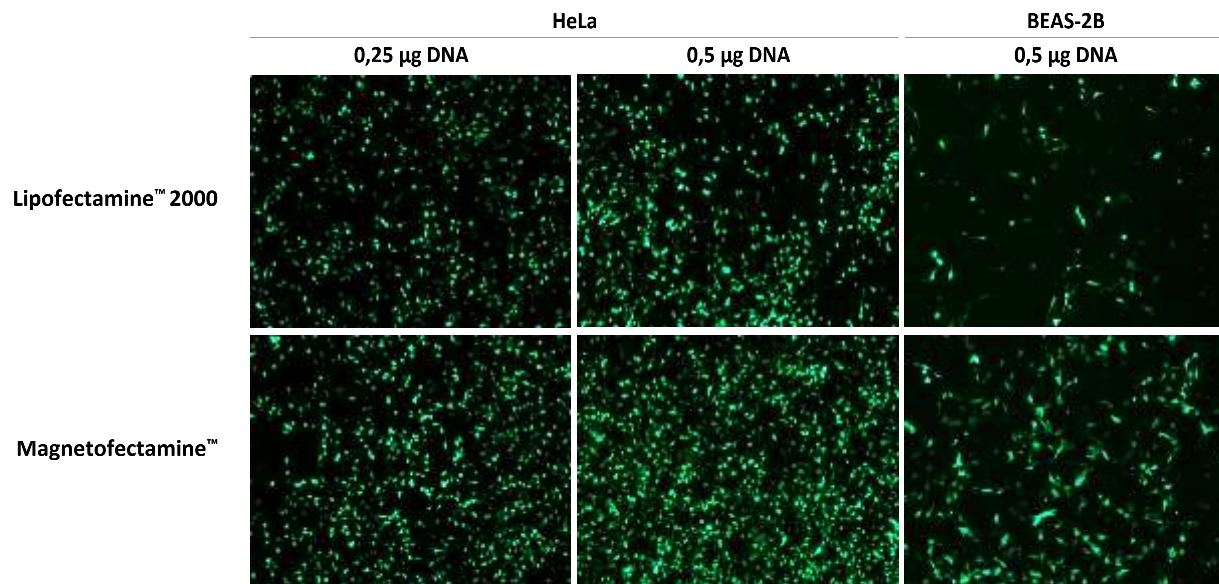




Cytotoxicity of Magnetofectamine™ versus Electroporation. Various primary cells were electroporated or transfected with Magnetofectamine™. 24 hours post-transfection, % of living cells was analyzed by FACS and MTT assay.

Optimization of DNA amount

Please refer to Magnetofectamine™ general protocol for more information on optimization parameters.



HeLa and BEAS-2B cells were transfected with either Lipofectamine™ 2000 alone according to the manufacturer protocol or with Magnetofectamine™ in a 24-well plate using 0.25 g and 0.5 µg of DNA for HeLa and 0.5 µg DNA for BEAS-2B. GFP expression was assessed under fluorescent microscope 24H after transfection.

Our team has developed many cell type specific protocols with optimized transfection conditions. Please contact our technical support service to obtain these protocols: tech@ozbiosciences.com

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