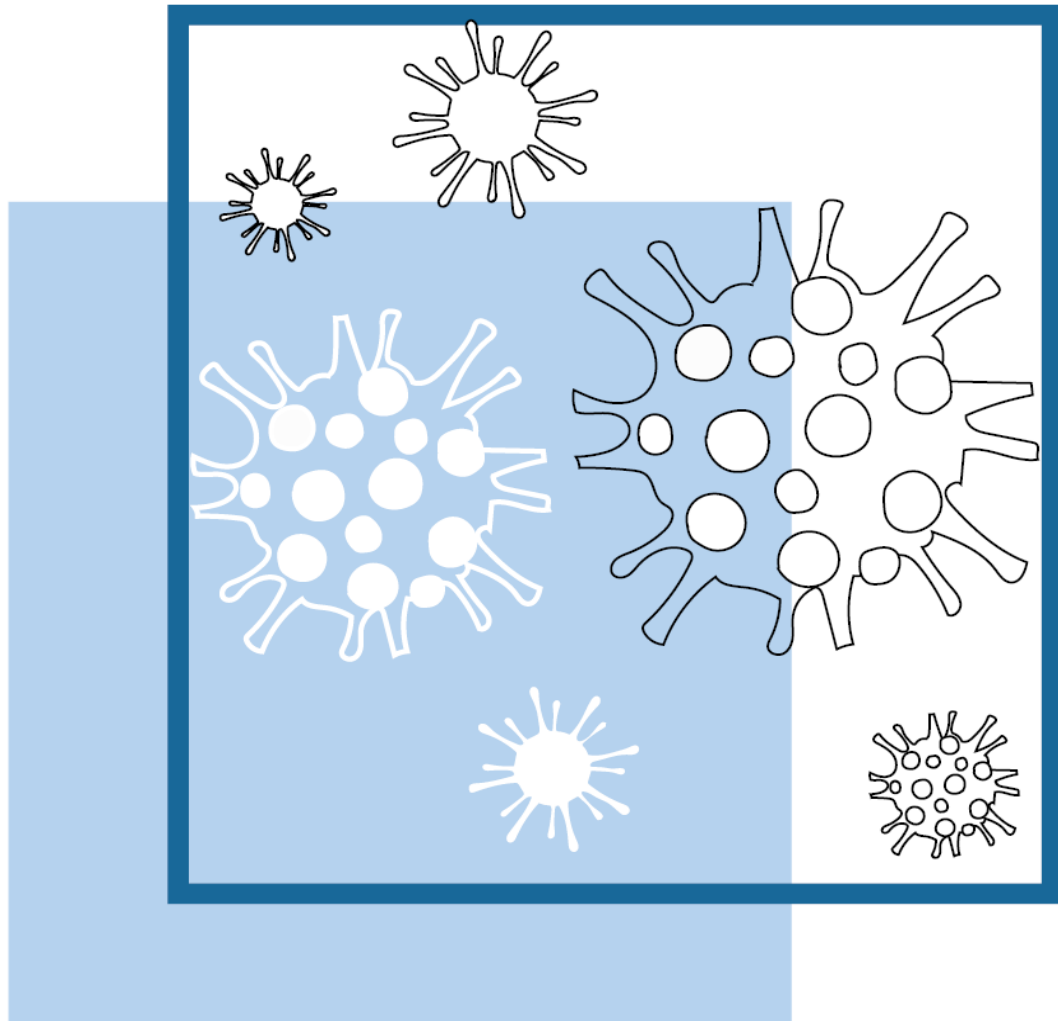


LentiBlast Premium Superior Grade

INSTRUCTION MANUAL



Instruction Manual

Description

LentiBlast Premium Superior Grade (LBP SG) The Lentiviral Transduction Enhancer

LentiBlast Premium Superior Grade is identical in synthesis and formulation to LentiBlast Premium Research use only Grade (RUO Grade) but provides more quality controls, batch validation and documentation as required for preclinical trials.

LentiBlast Premium Superior Grade (LBP SG) is a chemical lentiviral transduction enhancer, very effective for enhancing viral driven genetic modification. LentiBlast Premium Superior Grade is non-cytotoxic and improves transduction in a wide range of cell types from CD34+ hematopoietic Stem cells to primary cells or cell lines such as HT1080¹ or SH-SY5². Moreover, its formulation ensures total compatibility and performance for enhancing infection and transduction in CD4+ and CD8+ T lymphocytes^{3,4} and is perfect for CAR-T therapy⁵. The efficiency of LentiBlast Premium Superior Grade is such that the viral MOI (number of viral particles per cell) can be reduced and therefore fewer viruses can be used.

Catalog Number	Description	Volume (µL)	Number of transductions in a 24-well plate (average)
LBPSG15	LentiBlast Premium Superior Grade	1500 µL	300
LBPSG50	LentiBlast Premium Superior Grade	5000 µL	1000

For any technical question, contact us at tech@ozbiosciences.com

1. Technology

1.1. Description

LentiBlast Premium Superior Grade is the ideal reagent to enhance lentiviral infection and transduction in any type of cells: adherent or in suspension, primary or cell lines. Its patented chemical composition allows to simultaneously neutralize electrostatic repulsions between membrane and viral particles and to enhance viral fusion with cell membrane. Due to a favorable “membrane permeable effect” limiting the transmembrane potential changes, LentiBlast Premium Superior Grade is non-toxic.

NOTE: LentiBlast Premium Superior Grade can also be used with other enhancers and for sequential transductions.

¹ Arnoult N., Nature. 2017 (549), 548–552.doi:10.1038/nature24023

² Araújo-Vilar D., Eur J Hum Genet. 2018 Jan 24. doi: 10.1038/s41431-017-0052-8

³ Epstein AL., Patent, 2016

⁴ Benati D., J Clin Invest. 2016 Jun 1;126(6):2093-108.

⁵ Zheng L., Int J Mol Sci. 2017 Dec 20;18(12). pii: E2773

1.2. Storage and shipping condition

Storage: Upon reception and for long-term use, store the LentiBlast Premium Superior Grade transfection reagent at -20°C

Stability: 1 year upon receipt

Shipping condition: The reagent is shipped at RT

2. Applications and Protocols

2.1. General Considerations

- Allow reagent to reach room temperature before starting
- Do not change the transduction conditions already settled: simply add the LentiBlast Premium Superior Grade to your already established infection/transduction protocol
- If transduction conditions are unknown, we recommend beginning with a MOI of 2
- We suggest to first test 3 dilutions of LentiBlast Premium Superior Grade (1:1000, 1:100 and 1:50)

2.2. Cells preparation

Cell culture prior to transduction: the day before transduction prepare the cells according to the table below.

Effects of lentiviral transduction are generally observed after 48 to 96 h incubation. Cells should be 20-50 % confluent at the time of transduction (see the suggested cell number in the Table 1).

Tissue culture dish format	Surface area per well ¹	Cell number	Recommended volume for culture	Recommended volumes for viral preparation
96-well plate	0.3 cm ²	3 - 8 x 1.10 ³	100 µL	50 µL
24-well plate	2 cm ²	2 - 4 x 1.10 ⁴	500 µL	50 µL
12-well plate	4 cm ²	4 - 8 x 1.10 ⁴	1 mL	100 µL
6-well plate	9 cm ²	1 - 2 x 1.10 ⁵	2 mL	200 µL

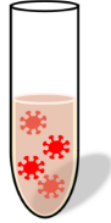
¹ Surfaces area may vary depending on the manufacturer.

Table 1: Recommended cell number and volume for viral/LentiBlast Premium complexes preparation

2.3. Standard Protocol

Use the following protocol to find the ideal conditions for LentiBlast Premium Superior Grade in 24-well plate. Adjust volumes to different well formats according to Table 1. If the lentiviral transduction/infection conditions are unknown, we recommend starting with a MOI of 2 using a lentiviral vector encoding for a fluorescent protein.

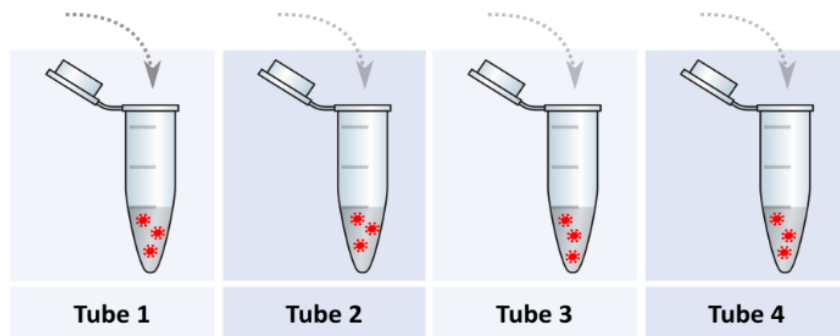
1. VIRUS PREPARATION



Dilute virus into culture medium sufficient for 4 samples (50 μ L each).

MOI 2 is recommended in case of unknown lentiviral transduction conditions.

2. DISPATCH EQUAL VOLUME OF VIRAL SUSPENSION INTO 4 TUBES



3. ADD LENTIBLAST TO EACH TUBE

	Tube 1	Tube 2	Tube 3	Tube 4
LentiBlast	-	0.5 μ L	5 μ L	10 μ L

4. MIX VIALS BY INVERTING

Do not vortex or centrifuge

5. ADD VIRUS +/- LENTIBLAST

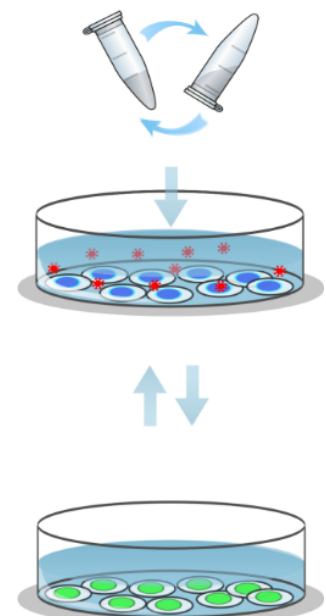
Incubate the cells 24 h under standard culture conditions

6. Optional: 24 h MEDIUM EXCHANGE

Remove medium from the cells
add pre-warmed culture medium

7. INCUBATE CELLS 24 TO 96 h.

Incubate the cells under standard culture conditions
We recommend performing assay from 24 to 96 h.



2.4. Optimization protocol

Depending on cell type (primary/cell lines, adherent/suspension, ...), virus serotype, gene of interest, culture conditions, readout..., transduction or infection efficiencies may vary. It may be necessary to optimize the conditions by (1) varying LentiBlast Premium Superior Grade amounts, (2) finely tuning transduction parameters or (3) centrifuging the cells.

2.4.1. Optimizing LentiBlast Premium volume

Using a fixed MOI, vary LentiBlast Premium Superior Grade volumes according to the Table 2 below:

Tissue culture dish format	1:1000	1:500	1:250	1:100	1:50	1:25
96-well plate	0.15 μ L	0.3 μ L	0.6 μ L	1.5 μ L	3 μ L	6 μ L
24-well plate	0.5 μ L	1 μ L	2 μ L	5 μ L	10 μ L	20 μ L
12-well plate	1 μ L	2 μ L	4 μ L	10 μ L	20 μ L	40 μ L
6-well plate	2 μ L	4 μ L	8 μ L	20 μ L	40 μ L	80 μ L

Table 2: Recommended dilutions and volumes of LentiBlast Premium depending on tissue culture dish format

2.4.2. Finely tuning transduction parameters

We have observed that replacing culture medium at the time of transduction with complete medium containing viral particles/ LentiBlast Premium Superior Grade could improve the overall efficiency.

- 1) Seed the cells as previously described
- 2) Prepare a viral suspension in complete culture medium; volume should correspond to cell culture volume (refer to table 1 for recommended volumes)
- 3) Add LentiBlast Premium Superior Grade to the viral suspension
- 4) Mix vial by inverting
- 5) Replace cell culture medium with viral suspension/ LentiBlast Premium Superior Grade solution
- 6) Optionally perform a medium change after 24H, and incubate 24H to 96H

2.4.3. Raising efficiency using Centrifugation step

For hard-to-transduced cells, a centrifugation step can be added to the standard protocol. Cells are prepared the day of transduction, counted, pelleted and suspended in Lentivirus/ LentiBlast Premium Superior Grade mix before centrifugation.

NOTE: Centrifugation may negatively influence cell viability.

- 1) Detach cells and seed them into 4 wells. Refer to Table 1 for suggested cell density
- 2) Follow steps 1 to 4 of the standard protocol and add lentivirus/ LentiBlast Premium Superior Grade mixes to cells
- 3) Centrifuge the plate 900 rpm for 90 min
- 4) Incubate cells overnight and proceed to steps 6 and 7 of the standard protocol

3. Quality controls

To assure the performance of each lot of LentiBlast Premium Superior Grade (LBP SG) and LentiBlast Premium (LBP) produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify function, quality and performance activity of each batch.

Quality Controls common to both LBP SG and LBP batches:

Quality Controls	Specification
Sterility	No fungal and bacterial contamination after 14 days with Thiglycolate assay
Biological activity	Lentiviral transduction enhancement on KG1a CD34+ cell line.

Quality Controls specific to each batch of LBP SG:

Quality Controls	Specification
Mycoplasma detection	No mycoplasma shall be detected*
Bacterial Endotoxin detection	≤ 1 EU/mL**
Chemical characterization	Identification of signature peaks by ¹ H and ¹³ C NMR
Purity	Single peak observed in SEC chromatogram

* Performed per United States Pharmacopeia guidelines: USP<63>

** Performed per United States Pharmacopeia guidelines: USP<85>

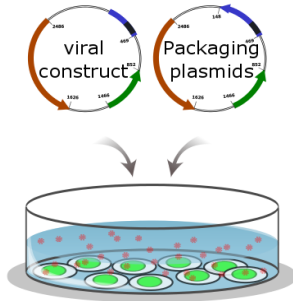
4. Troubleshooting

Problems	Comments and Suggestions
Low transduction efficiency	<p>1- MOI used is low. Raise the lentiviral particles amount; up to MOI 100 can be used.</p> <p>2- Infectious viral titer is low. Check viral titer via transduction of HEK-293T cells with serial dilutions of lentivirus.</p> <p>3- Cells are difficult to transduce. Use the centrifugation protocol (refer to paragraph 2.4.3).</p> <p>4- No effect of LentiBlast Premium Superior Grade is observed. Concentrations of LentiBlast Premium is too low or not optimized (refer to paragraph 2.4.1).</p>
Low viability	<p>1- MOI is too high. Lower the lentiviral dose</p> <p>2- LentiBlast Premium Superior Grade concentration is too high. Decrease concentration of LentiBlast Premium, refer to Table 2.</p> <p>3- Cells are sensitive to lentiviral treatment. Perform a medium change after 4 to 6 h or directly after the centrifugation process.</p>

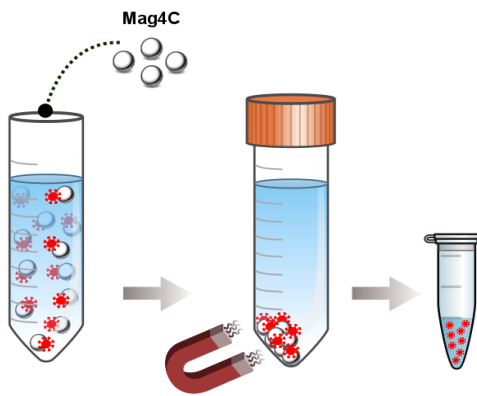
5. Related products

APPLICATIONS

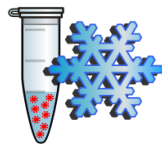
Viral Production



Capture / Concentration

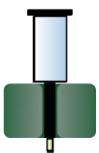


Conservation

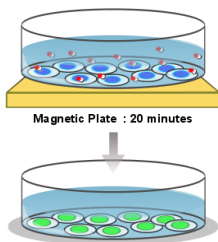


Infection Enhancement

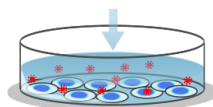
cell sorting
column



Magnetofection



BioChemicals



RECOMMENDED PRODUCTS

Calcium Phosphate
Transfection Kit

DreamFect Gold
Helix-IN Transfection Reagent

High transfection efficiency
High compaction level
Biodegradable

Mag4C Kits
Mag4C-Lv
Mag4C-Ad

Concentrate virus
Raise viral dose
Isolate virus from inhibitor
Conserve the viral particles

Mag4C
conservation buffer

Adenoviral particles
Retro/lentiviral particles

Magnetic Particles

Viro-MICST
ViroMag
AdenoMag

Raise transduction efficiency
Synchronize infection

BioChemicals

LentiBlast
AdenoBlast

Enhance transduction
Non toxic
Low MOI

Our technical support will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our latest breakthrough technologies and update:

<http://www.ozbiosciences.com>

Purchaser Notification

Limited License

The purchase price paid for LentiBlast Premium Superior Grade by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the section 1, Kit Contents). These reagents are intended **for internal research only** by the buyer. Such use is limited to the use in the product manual. Furthermore, research and preclinical only use means that this kit 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.

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Product Use Limitations

The LentiBlast Premium Superior Grade is developed, designed, intended, and sold for research and preclinical studies only. It is not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

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