LentiBlast Premium

INSTRUCTION MANUAL

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Lentivirus Transduction Enhancer

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The art of delivery systems

Instruction Manual

Description

LentiBlast Premium
The Lentiviral Transduction Enhancer

LentiBlast Premium is a chemical lentiviral transduction enhancer, very effective for enhancing viral driven genetic modification. LentiBlast Premium 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 HT1080[1] or SH-SY5[2]. 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 therapy[5]. The efficiency of LentiBlast Premium 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)
LBPX500	LentiBlast Premium 100 transductions	500 µL	100
LBPX1500	LentiBlast Premium 300 transductions	1500 µL	300

1. Technology

1.1. Description

LentiBlast Premium 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 is non-toxic.

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

1.2. Storage and shipping condition

Storage: Upon reception and for long-term use, store the LentiBlast Premium transfection reagent at -20°C
Stability: 1 year
Shipping condition: The reagent is shipped at RT

[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

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 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 (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

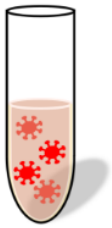
[1] Surfaces area may vary depending on the manufacturer.

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 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.

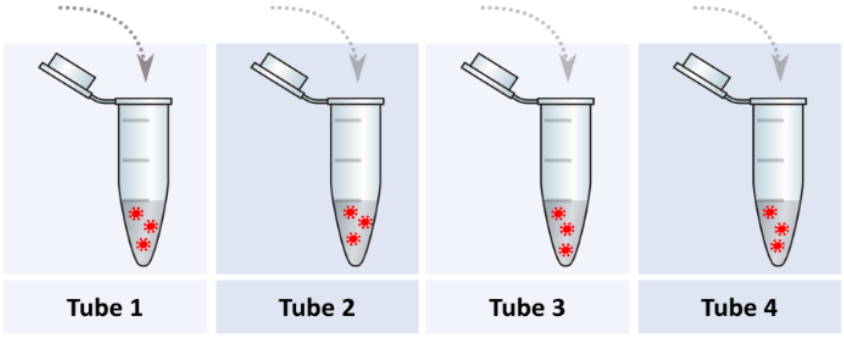
1. VIRUS PREPARATION



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

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

2. DISPATCH EQUAL VOLUME OF VIRAL SUSPENSION INTO 4TUBES



3. ADD LENTIBLAST TO EACH TUBE

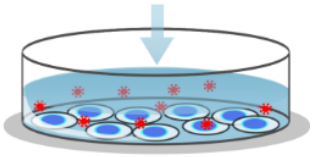
	Tube 1	Tube 2	Tube 3	Tube 4
LentiBlast	-	0.5 µL	5 µL	10 µL

4. MIX VIALS BY INVERTING

Do not vortex or centrifuge

5. ADD VIRUS +/- LENTIBLAST

Incubate the cells 24 h under standard culture conditions

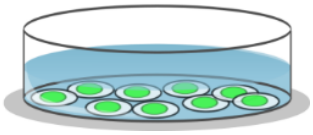


6. Optional: 24 h MEDIUM EXCHANGE

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 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 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 µL	0.3 µL	0.6 µL	1.5 µL	3 µL	6 µL
24-well plate	0.5 µL	1 µL	2 µL	5 µL	10 µL	20 µL
12-well plate	1 µL	2 µL	4 µL	10 µL	20 µL	40 µL
6-well plate	2 µL	4 µL	8 µL	20 µL	40 µL	80 µL

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 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 to the viral suspension
4) Mix vial by inverting
5) Replace cell culture medium with viral suspension/LentiBlast Premium 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 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 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. Troubleshooting

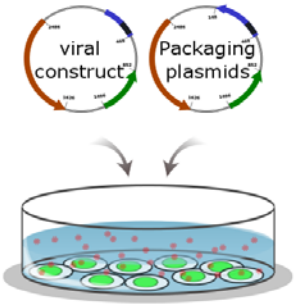
Problems	Comments and Suggestions
Low transduction efficiency	1- **MOI used is low.** Raise the lentiviral particles amount: up to MOI 100 can be used. 2- **Infectious viral titer is low.** Check viral titer via transduction of HEK-293T cells with serial dilutions of lentivirus. 3- **Cells are difficult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

4. Related products

APPLICATIONS

RECOMMENDED PRODUCTS

Viral Production

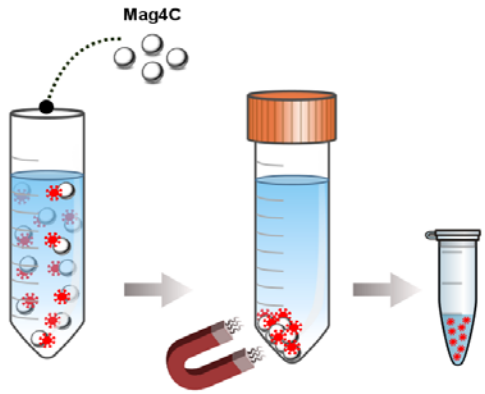


Calcium Phosphate Transfection Kit

DreamFect Gold
Helix-IN Transfection Reagent

- High transfection efficiency
- High compaction level
- Biodegradable

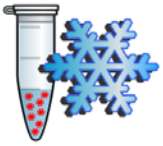
Capture / Concentration



Mag4C Kits
- Mag4C-Lv
- Mag4C-Ad

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

Conservation



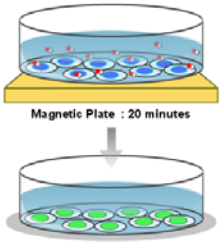
Mag4C
conservation buffer

- Adenoviral particles
- Retro/lentiviral particles

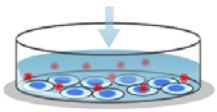
Infection Enhancement

cell sorting column

Magnetofection



BioChemicals



Magnetic Particles

Viro-MICST
ViroMag
AdenoMag

- Raise transduction efficiency
- Synchronize infection

BioChemicals

LentiBlast
AdenoBlast

- Enhance transduction
- Non toxic
- Low MOI