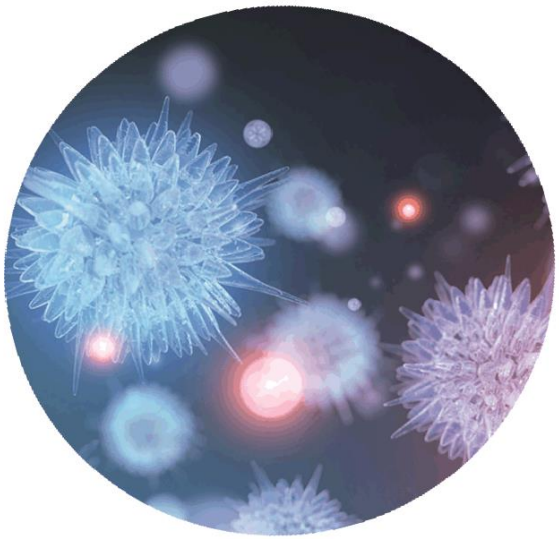


Viral Applications

LentiBlast Premium

Transduction reagent

Enhance lentiviral infection and tranduction efficiency

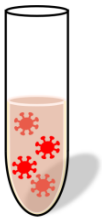
Protocol

OZBIOSCIENCES
The art of delivery systems

LentiBlast Premium Quick Protocol

Use the following protocol to find the ideal conditions for LentiBlast Premium in 24-well plate. 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.
NOTE: We suggest to use 0.5 to 10 µL of LentiBlast Premium per conditions. For hard-to-transduce cells, you can add a centrifugation step to this protocol.

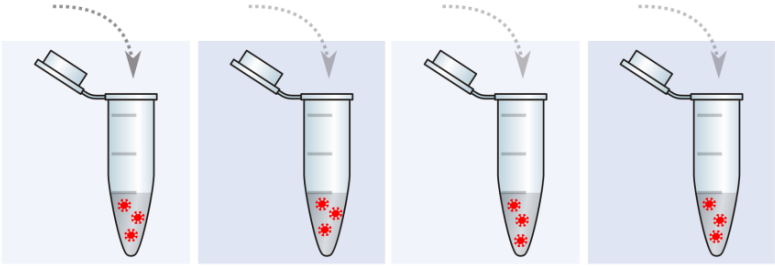
1. VIRUS PREPARATION



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

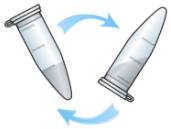


3. ADD LENTIBLAST TO EACH TUBE

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

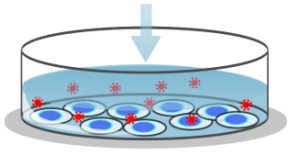
4. MIX VIALS BY INVERTING

Do not vortex or centrifuge



5. ADD VIRUS +/- LENTIBLAST

Incubate the cells 24 h under standard culture conditions

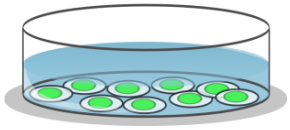


6. Optional: 24 h MEDIUM EXCHANGE

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.



IMPORTANT NOTES – Before you begin

- ✓ Allow reagents to reach RT and gently vortex them before utilization.
- ✓ Effects of lentiviral transduction are generally observed after 48 to 96 h.
- ✓ For hard-to-transduce cells, you can add a centrifugation step to this protocol.
- ✓ Dilute your reagent with deionized water for doses less than 1µL.
- ✓ If transduction conditions are unknown, we recommend beginning with a MOI of 2.
- ✓ Do not use LentiBlast Premium with another viral enhancer or adjuvant.
- ✓ In case of low efficiency using the standard protocol, transduction efficiency can be raised:

    a. **by centrifugation:** However, this procedure is not recommended because in some cases it can lower the viability. Please refer to Centrifugation Protocol below for more details.
    b. **by optimization:** Variations in MOI, volumes of LentiBlast Premium can lead to higher transduction efficiency.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

Package content	LBPX500: 500 µL of LentiBlast Premium Reagent LBPX1500: 1500 µL of LentiBlast Premium Reagent
Shipping conditions	Room Temperature
Storage conditions	Store the LentiBlast Premium Reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	LentiBlast Premium is ideal to enhance lentiviral infection and transduction in any type of cells: adherent or in suspension, primary or cell lines. Highly recommended for Stem cells
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells Preparation

Cell culture prior to transduction: the day before transduction prepare the cells according to the table below. Cells should be 20-50 % confluent at the time of transduction (see the suggested cell number in the Table 1).

Tissue Culture Dish	Cell Number
96 wells	3 – 8 x 1.10^3
24 wells	2 – 4 x 1.10^4
6 wells	1 – 2 x 1.10^5

Table 1: Suggested cell number for lentiviral transduction (per well)

IMPORTANT NOTE

For hard-to-transduce or non-permissive cells, prepare the cells the day of transduction and then refer to Centrifugation Protocol.

2. Standard Protocol

Use the quick protocol above to find the ideal conditions for LentiBlast Premium in 24-well plate.
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.

NOTE: We suggest using 0.5, 5 µL and 10 µL of LentiBlast Premium per conditions.

3. Centrifugation Protocol

For hard-to-transduce cells, it is recommended to add a centrifugation step to the standard protocol. Cells are prepared the day of transduction, counted, pelleted and suspended in Lentivirus/LentiBlast Premium mixes.

NOTE: Centrifugation may influence cell viability.

1) Detach cells and seed them into 5 wells. Refer to Table 1 for suggested cell density
2) Follow steps 1 to 4 of the quick 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 7 and 8 of the standard protocol

Optimization Protocol

1. Optimizing LentiBlast Premium volumes

To find the ideal transduction conditions using LentiBlast Premium, we recommend optimizing volumes of LentiBlast Premium with a fixed MOI (refer to Table 2).

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. 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

NOTES

Additional products for Viral Transduction Enhancement

- **ViroMag** for enhancing viral transduction efficiency (suitable for all viruses)
- **ViroMag R/L** for enhancing Lentiviral and Retroviral transduction efficiency
- **AdenoMag** specific for Adenoviral and AAV transduction

Additional products for Virus Capture and Concentration

- **Mag4C-LV** for Lentiviruses
- **Mag4C-AD** for Adenoviruses

Purchaser Notification

Limited License
The purchase of the LentiBlast Premium reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents) for the sole purpose of in-house research only, provided that no license, right or permission is granted hereunder to a non-academic, for-profit or commercial Licensee to use the LentiBlast Premium reagent for *ex vivo* gene therapy for hemoglobinopathies. The license does not include the use for any commercial or development purpose, including but not limited to any use for a) manufacturing, production, quality control, b) providing services, information or data, c) therapeutic, diagnostic, vaccine or prophylactic purposes or d) any applications which require regulatory approval as well as e) any clinical activities *in vivo* or *ex vivo*. The licensed use is limited to transfection of nucleic acids as described in the product manual. In addition, research 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 reagent and all of its components are developed, designed, intended, and sold for research use only. They are 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 use of the kit components by following proper research laboratory practices.

EUROPE & ASIA
OZ Biosciences SAS

163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
France

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

USA & CANADA
OZ Biosciences INC

4901 Morena Blvd
Suite 901
San Diego CA 92117
USA

Ph: + 1-858-246-7840
Fax: + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

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