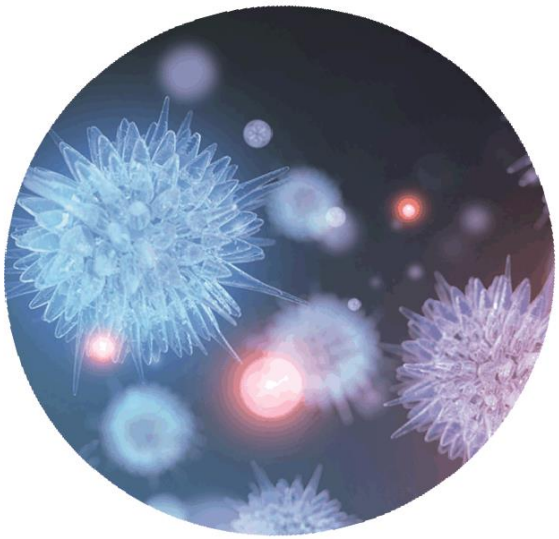

Viral Applications

# LentiBlast Premium

Transduction reagent

Enhance lentiviral infection and tranduction efficiency

# Protocol

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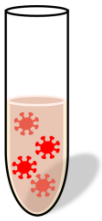
# LentiBlast Premium Quick Protocol

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.

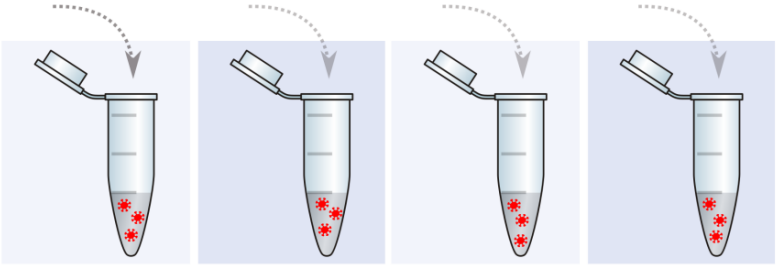
## 1. VIRUS PREPARATION



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

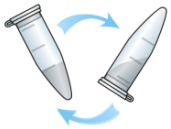



## 3. ADD LENTIBLAST TO EACH TUBE

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

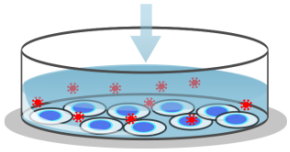
## 4. MIX VIALS BY INVERTING

Do not vortex or centrifuge



## 5. ADD VIRUS +/- LENTIBLAST

Incubate the cells 24 h under standard culture conditions



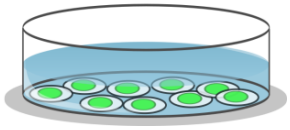
## 6. Optional: 24 h MEDIUM EXCHANGE

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.

| | |
|---|---|
| Package content | LBPX500: 500 µL of LentiBlast Premium Reagent<br>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

## 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