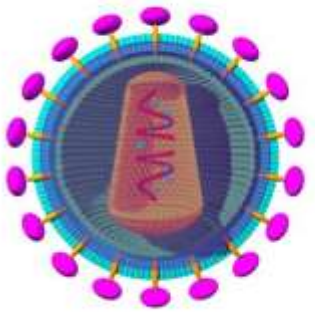
# LentiBlast

# INSTRUCTION MANUAL

***Lentivirus Transduction Enhancer***



LentiBlast

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# LentiBlast

## Instruction Manual

**LentiBlast** is the ideal Lentiviral Transduction Enhancer.

List of LentiBlast kits

| Catalog Number | Description | Volume (µL) | Number of transduction in a 24-well plate |
|---|---|---|---|
| LB00500 | LentiBlast 100 transductions | 500 µL – solution A<br>500 µL – solution B | 100 |
| LB01500 | LentiBlast 300 transductions | 1500 µL – solution A<br>1500 µL – solution B | 300 |

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (order@ozbiosciences.com). For all other supplementary information, do not hesitate to contact our dedicated technical support (tech@ozbiosciences.com).


**OZ Biosciences SAS**
163 avenue de Luminy
Case 922, zone entreprises
13288 Marseille cedex 09 - France
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

**OZ Biosciences INC**
4901 Morena Blvd
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

# 1. Technology

## 1.1. Description

**LentiBlast** is the ideal reagent developed to enhance lentiviral infection and transduction in any type of cells, adherent or in suspension, primary or cell lines. Its patent-protected chemical composition allows simultaneously neutralizing electrostatic repulsions between membrane and viral particles and enhancing viral fusion with cell membrane. Due to a favorable "membrane permeable effect" limiting the transmembrane potential changes, **LentiBlast** is non-toxic.
NOTE: LentiBlast can also be used with other enhancers and for sequential transductions.

**LentiBlast** benefits:

- Enhances infection and transduction efficiency of lentivirus
- Non-toxic
- Compatible with cell lines and primary cells
- Allows using reduced amounts of Lentivirus (low MOI)
- Composed of two reagents for a higher compatibility and efficiency

## 1.2. Kit Contents, Stability and Storage

**Contents**
Kits content varies according to their size:
LentiBlast 100 transductions:

- 1 tube containing 500 µL of LentiBlast reagent A good for up to 100 transductions in a 24-well plate.
- 1 tube containing 500 µL of LentiBlast reagent B good for up to 100 transductions in a 24-well plate.

LentiBlast 300 transductions:

- 1 tube containing 1500 µL of LentiBlast reagent A good for up to 300 transductions in a 24-well plate.
- 1 tube containing 1500 µL of LentiBlast reagent B good for up to 300 transductions in a 24-well plate.

**Storage and Shipping**

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

Shipping condition: Room Temperature

# 2. Applications

**LentiBlast** has been specifically developed for enhancing lentiviral transduction in any type of cell, adherent or in suspension, primary or cell lines. **LentiBlast** is composed of two reagents **–A** and **–B** to offer the best combination for a specific transduction, and in order to get highest transduction efficiency as well as lowest toxic effect.

# 3. General Protocol

## 3.1. General Information / Important Guidelines

The **LentiBlast** is composed of two reagents: **LentiBlast –A** and **–B**. The best combination of these two solutions has to be found in a first step to get the best efficiency/viability ratio. We recommend beginning with two concentrations of each solution: **1:1000** and **1:100**.

Do not change the transduction conditions already settled: simply add the LentiBlast to your already established protocol. If transduction conditions are unknown, we recommend beginning with a MOI of 2.

In case of low efficiency using the standard protocol, transduction efficiency can be raised:

- by centrifugation. However this procedure is not recommended because in some cases it can lower the viability. Please refer to centrifugation protocol (paragraph 3.5) for more details.
- by optimization. Variations in MOI, volumes of LentiBlast –A and –B can lead to higher transduction efficiency.

## 3.2. 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. Cells should be 20-50 % confluent at the time of transduction (see the suggested cell number in the Table 1).

**Table 1**: Cell number suggested for lentiviral transduction (per well).

| Tissue Culture Dish format | Surface area per well[1] | Cell Number |
|---|---|---|
| 96 wells | 0.3 $cm^2$ | 3 – 8 x $1.10^3$ |
| 24 wells | 2 $cm^2$ | 2 – 4 x $1.10^4$ |
| 6 wells | 10 $cm^2$ | 1 – 2 x $1.10^5$ |

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

**Note:** For hard-to-transduce or non-permissive cells, prepare the cells the day of transduction and then refer to §3.4 (Centrifugation Protocol)

## 3.3. Standard Protocol

Use the following protocol to find the ideal conditions for LentiBlast 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.

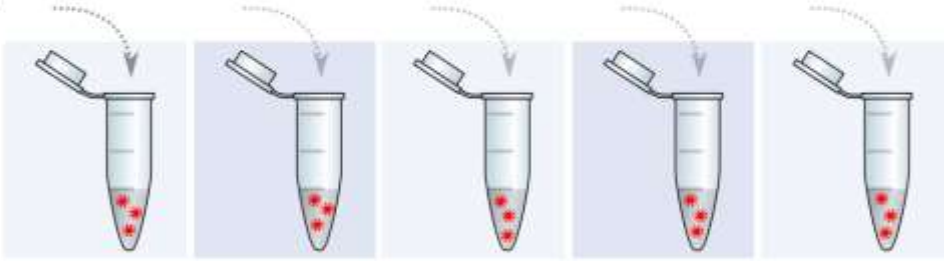
**Note:** We suggest to use **0.5 and 5 µL of LentiBlast –A and –B per conditions.**

### 1. VIRUS PREPARATION

Dilute virus into complete culture medium sufficient for 5 samples.

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

### 2. DISPATCH EQUAL VOLUME OF VIRAL SUSPENSION INTO 5 TUBES



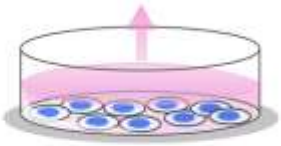

### 3. ADD LENTIBLAST -A & -B TO EACH TUBE

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

### 4. MIX VIALS BY INVERTING

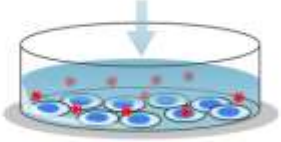
Do not vortex or centrifuge

### 5. ASPIRATE MEDIUM FROM CELLS



### 6. ADD VIRUS +/- LENTIBLAST -A/-B

Incubate the cells 24 h under standard culture conditions

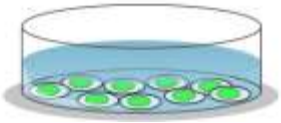


### 7. CHANGE MEDIUM

Remove medium from the cells and add pre-warmed culture medium

### 8. INCUBATE CELLS 24 TO 96 h.

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



## 3.4. Centrifugation Protocol

For hard-to-transduced 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 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 standard protocol and add lentivirus/lentiBlast 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.

## 3.5. Optimization

To find the ideal transduction conditions using LentiBlast, we recommend optimizing volumes of LentiBlast –A and –B using a MOI of 2 and 10.

**Table 3**: Recommend amounts of LentiBLast for optimization procedure.

| | MOI | LentiBlast Solution | | Example 24-well (500µL) | |
|---|---|---|---|---|---|
| | | LentiBlast –A | LentiBlast –B | LentiBlast –A | LentiBlast –B |
| #1 | 2 | 1:100 | 1:100 | 5 µL | 5 µL |
| #2 | 2 | 1:100 | - | 5 µL | - |
| #3 | 2 | - | 1:100 | - | 5 µL |
| #4 | 2 | 1:1000 | 1:1000 | 0.5 µL | 0.5 µL |
| #5 | 2 | 1:1000 | - | 0.5 µL | - |
| #6 | 2 | - | 1:1000 | - | 0.5 µL |
| #7 | 2 | 1:100 | 1:1000 | 5 µL | 0.5 µL |
| #8 | 2 | 1:1000 | 1:100 | 0.5 µL | 5 µL |
| #9 | 10 | 1:100 | 1:100 | 5 µL | 5 µL |
| #10 | 10 | 1:100 | - | 5 µL | - |
| #11 | 10 | - | 1:100 | - | 5 µL |
| #12 | 10 | 1:1000 | 1:1000 | 0.5 µL | 0.5 µL |
| #13 | 10 | 1:1000 | - | 0.5 µL | - |
| #14 | 10 | - | 1:1000 | - | 0.5 µL |
| #15 | 10 | 1:100 | 1:1000 | 5 µL | 0.5 µL |
| #16 | 10 | 1:1000 | 1:100 | 0.5 µL | 5 µL |
| #17 | 10 | - | - | - | - |

# 4. Appendix

Our dedicated and specialized technical support team will be pleased to answer any of your requests at tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com.

## 4.1 Quality Controls

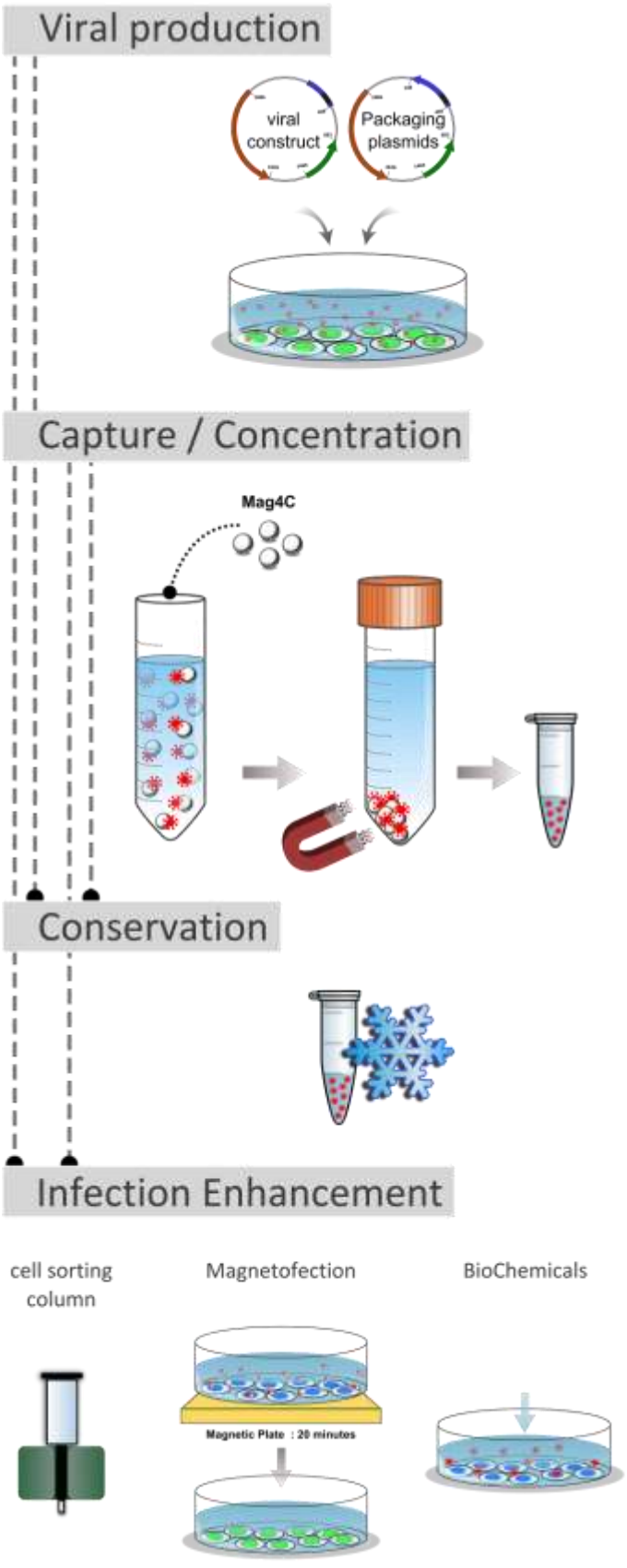
To assure the performance of each lot of LentiBlast reagent produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each component.

| Specification | Standard Quality Controls |
|---|---|
| *Sterility* | Thioglycolate assay. Absence of contamination shall be obtained for 15 days. |
| *Biological Activity* | Transduction efficiency on NIH-3T3 cells. Every lot shall have an acceptance specification of > 90% of the activity of the reference lot. |

## 4.2. 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.<br>2- **Infectious viral titer is low.** Check viral titer via transduction of HEK-293 cells with serial dilutions of lentivirus.<br>3- **Cells are difficult to transduce.** Use the centrifugation protocol (refer to paragraph 3.4).<br>4- **No effect of LentiBlast is observed.** Concentrations of LentiBlast is too low or ratios of each solution –A and –B are not optimized (refer to paragraph 3.5). |
| Low viability | 1- **MOI is too high.** Lower the lentiviral dose<br>2- **LentiBlast concentration is too high.** Decrease concentration of LentiBlast –A or –B, refer to table 3.<br>3- **Cells are sensitive to lentiviral treatement.** Perform a medium change after 4 to 6 h or directly after the centrifugation process. |

## 5. Related Products




### Calcium Phosphate transfection Kit

**DreamFect Gold**

- 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

### i-MICST: ViroMICST

Magnetic

**ViroMag**
**ViroMag R/L**
**AdenoMag**

- viral concetration onto the cell surface
- 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.

## 6. Purchaser Notification

**Limited License**

The purchase of the LentiBlast 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 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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Director of Business Development
OZ Biosciences SAS
Parc Scientifique et Technologique de Luminy
163, avenue de Luminy, zone entreprise, case 922
13288 Marseille Cedex 9, France
Tel: +33 (0)4.86.94.85.16
Fax: +33 (0)4. 86.94.85.15
E-mail: business@ozbiosciences.com

# CONTACTS


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

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

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

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

**www.ozbiosciences.com**


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