LentiBOOST™ Transduction Enhancer

Quick Start Guide

Product number: LentiBOOST-P TE

Format: 100 mg/ml aqueous solution

Document version: 1

1. Product Description

LentiBOOST™ technology enhances the uptake of lentiviral vectors into mammalian cells. Compared to commonly used transduction enhancers like polybrene or protamine sulfate, it doesn't negatively affect cell viability or growth.

2. Expiration Date & Storage

Not to be used after expiration date. Store at -15°C to -25°C.

3. Thawing and handling

It is recommended to thaw LentiBOOST enhancer between +4°C and +25°C.
Before opening the tube, spin down briefly to remove any liquid from the lid. To keep for later use, you can aliquot LentiBOOST enhancer using aseptic technique and store at -15°C to -25°C. LentiBOOST transduction enhancer can be stored at +4°C for 1 month.
PLEASE NOTE: The protocols given below are suggestions only based on (published) customer data. Protocols must be adapted depending on customer-specific conditions.

4. Transduction Protocol for CD34+ Transduction

Preparation of human CD34+ HSC
CD34+ hematopoietic stem cells (HSC) are isolated from PBMC using standard protocols.

Day 1: Seeding cells
CD34+ HSC are cultured according to standard protocols. Cells are incubated at 37°C in a humidified incubator containing 5% CO2.

Day 2: Transduction

- The following day, CD34+ HSCs are counted and $1x10^6$ cells are seeded per well in a 24 well plate.
- LentiBOOST enhancer and lentiviral vector are directly added to the cells.
- For an initial experiment, it is recommended to use MOIs between 2 - 30 for transduction and to add LentiBOOST enhancer at an initial concentration of 1 mg/ml (1:100) of the total volume (medium + virus).
- In a second experiment, it is recommended to titrate LentiBOOST enhancer in the range of 5 mg/ml – 0.1 mg/ml (1:20-1:1000) to determine the minimal active concentration.
- Optionally spinoculation can be performed at 600g for 90 minutes at room temperature.

Day 3: Medium exchange
Aspirate medium from transduced cells and add appropriate amount of normal growth medium.

5. Transduction Protocol T-cells

Seeding and pre-stimulation of cells

Primary T Cells are stimulated according to standard protocols. This step should be adapted according to individual protocols.

Day 1: Transduction

- Thaw lentivirus at +4°C and prepare 500µl of culture medium.
- Add LentiBOOST enhancer at 1 mg/ml (1:100) as starting point. Concentrations of LentiBOOST enhancer can be tested between 0.1 mg/ml and 5 mg/ml.
- Add viral vector to medium at desired MOI and mix gently. For an initial experiment, it is recommended to use MOIs between 2 - 30 for transduction.
- Pellet 10^6 cells by centrifugation (cell number can be adapted according to needs). Mix the cell pellet with the prepared medium containing LentiBOOST enhancer and viral vector. Seed the cells into a 24 well plate.
- Optionally spinoculation can be performed at 800g for 90 minutes at room temperature.
- Incubate overnight at 37°C and 5% CO_2.

Day 2: Medium change

- Exchange medium according to standard protocols.

6. Troubleshooting

Here are some general approaches that LentiBOOST enhancer users have found useful when troubleshooting issues. Please note that users will need to account for the specific system.

Trouble	Possible reason	Solution
Low transduction efficiency	MOI used was too low	Use higher amounts of lentivirus up to MOI 50
	Cells are very hard to transduce	Include spinoculation step 800 g for up to 90 min at room temperature (in cell culture plates)
		Increase concentration of LentiBOOST enhancer up to 5 mg/ml (1:20)
		Add Protamine sulfate at 5 µg/ml additionally to LentiBOOST enhancer
Low viability	Cells are sensitive to LentiBOOST enhancer	Decrease concentration of LentiBOOST enhancer to e.g. 0.2 mg/ml, 0.1 mg/ml (1:500, 1:1000)
	Cells are sensitive to spinoculation	Try protocol without spinoculation Reduce duration Reduce velocity
	Cells are sensitive to lentiviral vectors	Change medium 4 h after transduction or directly after centrifugation

LentiBOOST Pharma-Grade technology: For research use only. Not for use in diagnostic procedures.
LentiBOOST GMP Grade technology: Not for diagnostic use. Other applications must be authorized by a license from Revvity.