MANUAL

LentiBOOST™

Transduction Enhancer

Part number: LentiBOOST™-P

Storage: -15°C to -25°C

Shipped at room temperature

Version: 1 Date: July 2024

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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 does not negatively affect cell viability or cell growth.

Furthermore, for human CD34+ hematopoietic stem cells (section 6) and progenitor cells studies have indicated that LentiBOOST transduction enhancer (TE) allows to maintain the differentiation potential of the cells after lentiviral transduction (Hauber et al. 2018). The authors observed high transduction rates while limiting the vector copy number (VCN close to 5) to a moderate increase that reaches each individual cell as requested by regulatory guidelines.

For human T-cells (section 7) a group of scientists has shown that the use of LentiBOOST TE technology for lentiviral transduction can result in a 2-fold increase in transduction rate, without observing a cytotoxic effect on the cells (Simon et al. 2019).

LentiBOOST technology is therefore an excellent tool for enhancing lentiviral transduction of sensitive primary cells including hematopoietic stem cells and T-cells.

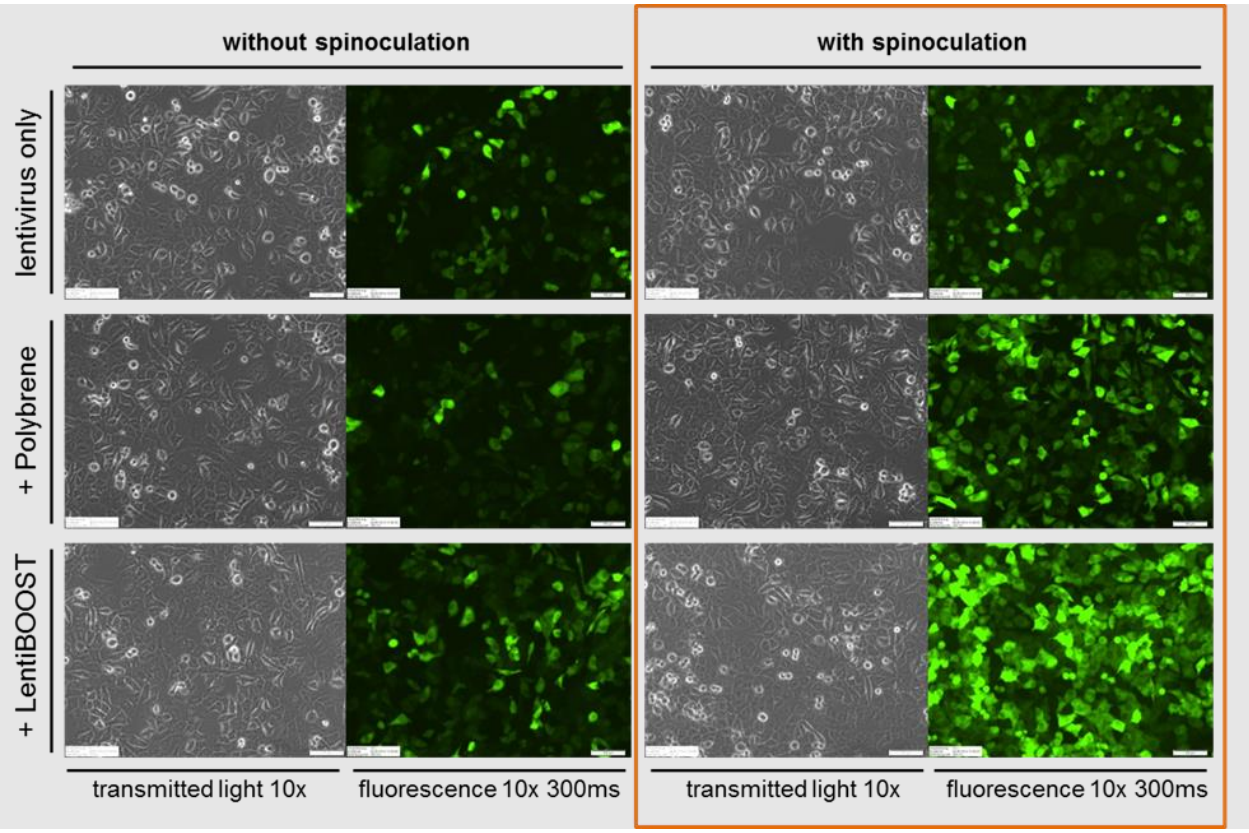

Fig. 1: Lentiviral transduction efficiencies in H1299 48h after transduction with lentivirus LV-CMV-GFP. Protocols according to the instructions of this manual.

2. LentiBOOST TE pharma grade non GMP

LentiBOOST-P TE 100 mg/ml aqueous solution

3. Expiration Date

Not to be used after expiration date.

4. Storage

Store at -15°C to -25°C.

5. 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 TE can be stored at +4°C for 1 month. No loss of functionality* was observed after 5 freeze-thaw cycles.

*based on an internal standardized assay, no conclusion can be drawn to any customer specific cell types.

PLEASE NOTE: The protocols given below are suggestions only based on (published) customer data. Protocols must be adapted depending on customer-specific conditions.

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6. 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 TE 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.

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7. 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.
- Prepare 500µl of culture medium and add LentiBOOST enhancer at 1 mg/ml (1:100) as starting point.
- Concentrations of LentiBOOST TE 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.
- Pellet 10^6 cells by centrifugation (cell number can be adapted according to needs).
- Mix the cell pellet with the prepared medium containing LentiBOOST TE and viral vector.
- Seed the cells into a 24 well plate.
- Spinoculation (optional): Centrifuge cell culture plate for 90 min at 800 g at RT.
- Incubate the overnight at 37°C and 5% CO_2.

Day 2: Medium change

- Exchange medium according to standard protocols.

8. Literature

Hauber, I., Beschorner, N., Schrödel, S., Chemnitz, J., Kröger, N., Hauber, J., & Thirion, C. (2018). Improving Lentiviral Transduction of CD34+ Hematopoietic Stem and Progenitor Cells. Human Gene Therapy Methods, hgtb.2017.085. https://doi.org/10.1089/hgtb.2017.085

Simon, B., Harrer, D. C., Thirion, C., Schuler-Thurner, B., Schuler, G., & Uslu, U. (2019). Enhancing lentiviral transduction to generate melanoma-specific human T cells for cancer immunotherapy. Journal of Immunological Methods, 472(April), 55–64. https://doi.org/10.1016/j.jim.2019.06.015

9. Troubleshooting

Here are some general approaches that LentiBOOST TE users have found useful when troubleshooting issues. However, 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 TE
Low viability	Cells are sensitive to LentiBOOST enhancer	Decrease concentration of LentiBOOST TE 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

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