SIRION BIOTECH

Product Manual

LentiBOOST®

Transduction Enhancer

Shipped at room temperature
Store at -20 °C

Revision 01/2022

FOR RESEARCH USE ONLY (LentiBOOST®-P)

www.sirion-biotech.com

1. *PRODUCT DESCRIPTION*

LentiBOOST® 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 and progenitor cells LentiBOOST® was shown to maintain the "stem-cell like" potential of the cells after lentiviral transduction (Hauber et al. 2018). Moreover, it shows high transduction rates while limiting the vector copy number (VCN < 5) that reaches each individual cell even at low MOI (< 10).

For human T-cells it was shown that the use of LentiBOOST® for lentiviral transduction results in a 2-fold increase in transduction rate, has no cytotoxic effect on the cells and does not affect cytokine production after antigen encounter (Simon et al. 2019).

Additionally, the use of LentiBOOST® can increase vector copy number (VCN) up to 4-fold (Hauber et al. 2018).

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

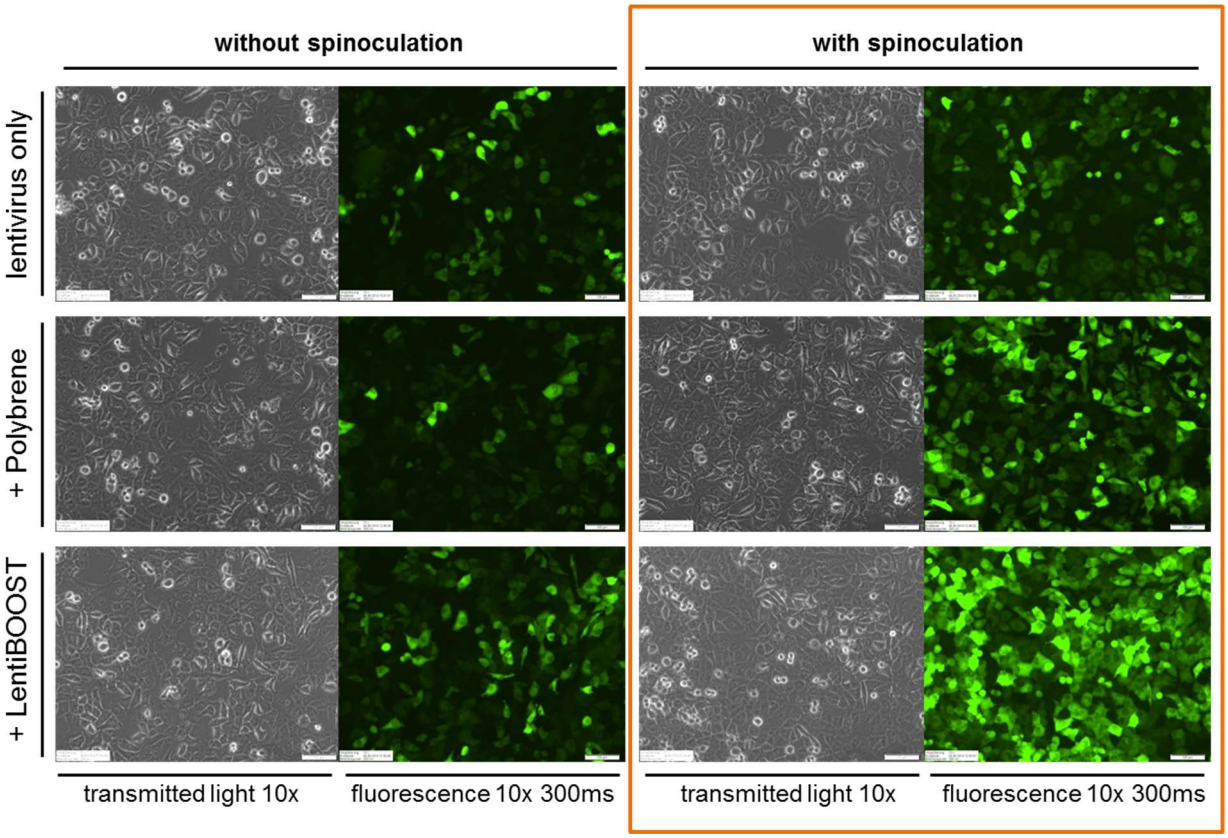

Fig. 1: Lentiviral transduction efficiencies in H1299 48h after transduction with lentivirus LV-CMV-GFP. Protocols according to the instructions of this manual

2. *Material*

LentiBOOST® 100 mg/ml aqueous solution

3. *Storage*

Store at -20°C.

4. *Thawing and handling*

It is recommended to thaw LentiBOOST® at room temperature <+25°C.

Before opening the tube spin down briefly to remove any liquid from the lid. For further use aliquot LentiBOOST®-P using aseptic technique and store at -20°C. LentiBOOST®-P 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

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PLEASE NOTE: the protocols given below are suggestions based on (published) customer data. Protocols have to be adapted depending on customer specific conditions.

5. *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 $1x10^6$ HSCs transduced in the presence of LentiBOOST® and Lentivirus particles in 24 well plates using MOI 10 (see note below). For an initial experiment it is recommended to use MOIs between 2-30 for transduction and to add LentiBOOST® at the standard concentration of 1 mg/ml (1:100) of the total volume (medium + virus). In a second experiment it is recommended to titrate LentiBOOST® in the range of 5 mg/ml – 0.1 mg/ml (1:20-1:1000) to determine the minimal active concentration.

Protocol steps:

- Calculate the volume of lentivirus needed (see Table 1)

				Amount of infectious lentiviral particles					
Plate	CD34 Cell number/well	Total Volume (Medium+Virus)	Volume LentiBOOST®	MOI 30	MOI 15	MOI 10	MOI 5	MOI 2	MOI 0
24 well	1.00E+06	1 ml	10 µl (1:100)	3E+06	1.50E+06	1.0E+06	5.0E+05	2.0E+05	0

Table 1: Recommended volumes of medium and LentiBOOST®-P to be used for lentiviral transductions in an initial experiment. It is recommended to titrate LentiBOOST®-P in a second experiment in order to determine the minimal active concentration. Therefore, we recommend to dilute LentiBOOST® in the range of 1:20-1:1000 (5 mg/ml – 0.1 mg/ml). For other multiwell plates parameters have to be adjusted accordingly.

- Thaw lentivirus at +4°C
- Add the appropriate amount of LentiBOOST® according to Table 1 directly to the cells seeded the day before
- Add the amount of lentivirus according to Table 1 directly to the cells and mix carefully
- Incubate cells over night at normal cell culture conditions

Day 3: Medium exchange

- Aspirate medium from transduced cells and add appropriate amount of normal growth medium

- *Note: The MOI was determined according to the following method: VSV-G pseudotyped lentiviral vector particles purified and concentrated by ultracentrifugation were resuspended in StemMACS™ HSC Expansion Media (Miltenyi Biotec), aliquoted and stored at -80°C.*
- *The titer of pseudotyped lentiviral particles was determined as fluorescence forming units per ml (ffu/ml). This required transduction of 293T cells grown in standard medium (DMEM 10 % FBS) in 24-well plates with different volumes of viral vectors in presence of 0.5 µg/ml protamine sulfate. The cells were then spinoculated at 600 x g for 10 min at room temperature. Medium was changed 8 h after transduction. At 72 h post transduction, cells were harvested and analyzed by flow cytometry for GFP expression. Samples that contained 5 to 25% GFP positive cells were used to calculate viral titers.*

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6. *Transduction Protocol T-cells*

Seeding and pre-stimulation of cells

- Primary T Cells are stimulated with anti-CD3 antibody, anti-CD28 antibody and interleukin-2 (IL-2) according to standard protocols. This step should be adapted according to individual needs.

Day 1: Transduction

- Thaw lentivirus at +4°C
- Prepare 500µl of culture medium and add LentiBOOST® at 1 mg/ml (1:100) as starting point. Concentrations of LentiBOOST® 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® 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

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7. *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

8. *Troubleshooting:*

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-P up to 5 mg/ml (1:20)
		add Protamine sulfate at 5 µg/ml additionally to LentiBOOST(R)
Low viability	Cells are sensitive to LentiBOOST®	Decrease concentration of LentiBOOST® 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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