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 **SIRION**
BIOTECH

Product Manual

LentiBOOST-P

Transduction Enhancer

Shipped at room temperature
Store at -20 °C

FOR RESEARCH USE ONLY

www.sirion-biotech.de

PRODUCT DESCRIPTION

LentiBOOST-P 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 it was shown that the use of LentiBOOST-P during lentiviral transduction maintains the “stem-cell like” potential of the cells. Moreover, it shows high transduction rates while limiting the vector copy number (VCN < 5) that reaches each individual cell even at low MOI (< 10).

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

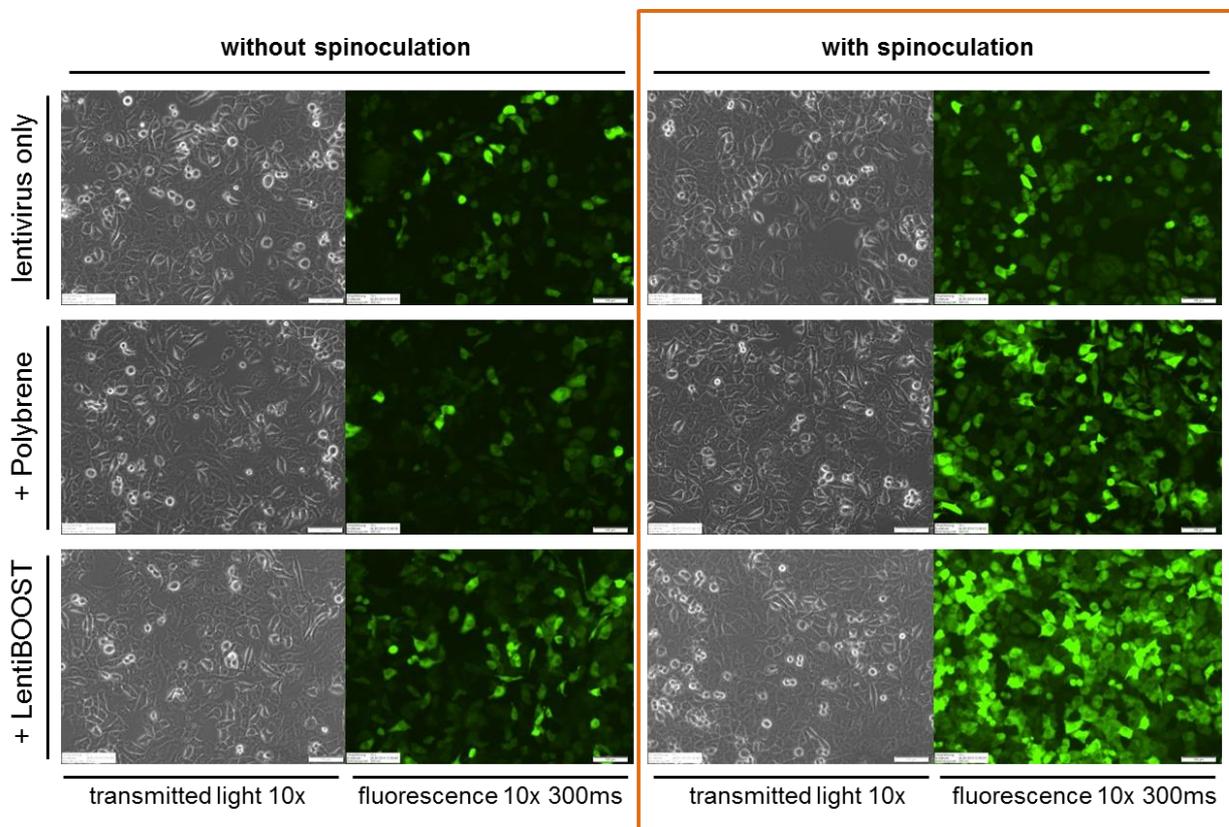


Fig. 1: Lentiviral transduction efficiencies in H1299 48h after transduction with lentivirus LV-CMV-GFP. Protocols according to the instructions of this manual

MATERIALS SUPPLIED

LentiBOOST-P 500 µl à 100 mg/ml or 1500µl à 100 mg/ml

STORAGE

Store at -20°C.

Transduction Protocol

Day 1: Seeding cells

For 6 well plates seed 1,00E+05 cells per well.

- *Note: If your cells are maintained under selective pressure, seed cells without selection antibiotics and maintain them without antibiotics until day 3.*

Day 2: Transduction

For an initial experiment it is recommended to use MOIs between 2-30 for transduction and to add LentiBOOST-P to the standard concentration of 1:100 of the total volume (medium+virus). In a second experiment it is recommended to titrate LentiBOOST-P in the range of 1:20-1:1000 to determine the minimal active concentration.

- Calculate the volume of lentivirus needed (see Table 1)
- Thaw lentivirus at 4°C
- Add the appropriate amount of LentiBOOST-P 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
- Spinoculate (optional): Centrifuge cell culture plate for 90 min at 800 g at RT.
 - *Note: Spinoculation has been shown to increase transduction efficiency for most cell types. Cells should always be centrifuged in cell culture plates to minimize shear stress.*
- Incubate cells over night at normal cell culture conditions

Plate	Cell number/well	Total Volume (Medium+Virus)	Volume LentiBOOST-P	Amount of infectious lentiviral particles					
				MOI 30	MOI 15	MOI 10	MOI 5	MOI 2	MOI 0
6 well	1,00E+05	2 ml	20 µl	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-P in the range of 1:20-1:1000. For other multiwell plates parameters have to be adjusted accordingly.

Day 3: Medium exchange

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

Day 4: Maintenance

- In case the cells do not tolerate to be grown at high confluence they can be split on day 4. For further cultivation exchange medium and passage cells at regular intervals.

Day 5: Generation of stable cell lines (optional)

If lentiviral vectors encoding for antibiotic selection markers are used, stable cell lines can be generated by selecting transduced cells. An optimal concentration of the selection antibiotic has to be determined in a separate experiment (killing curve).

- *Note: Always include a negative control in your lentivirus transduction experiment (non-transduced control).*
- Remove cell culture medium from cells
- Add medium containing the appropriate concentration of antibiotic.
- Culture cells in the presence of antibiotics and change medium in regular intervals.

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
		Increase volume of LentiBOOST-P up to 1:20
Low viability	Cells are sensitive to LentiBOOST-P	Decrease volume of LentiBOOST-P e.g. 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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