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SIRION BIOTECH

Product Manual

LentiBOOST™

Lentiviral Transduction Enhancer

SB-P-LV-101-01	100 standard transductions
SB-P-LV-101-02	300 standard transductions

Shipped at room temperature
Store at -20 °C

FOR RESEARCH USE ONLY

PRODUCT DESCRIPTION

LentiBOOST™ has been developed to enhance the uptake of lentiviral vectors into primary cells and cell lines. LentiBOOST™ contains two separate components acting synergistically, one by reducing electrostatic repulsion between the virion and cell membrane, the second increases membrane fusion.
LentiBOOST™ is especially recommended for cells, which are hard to transduce or which are sensitive to other transduction enhancers (e.g. Polybrene).

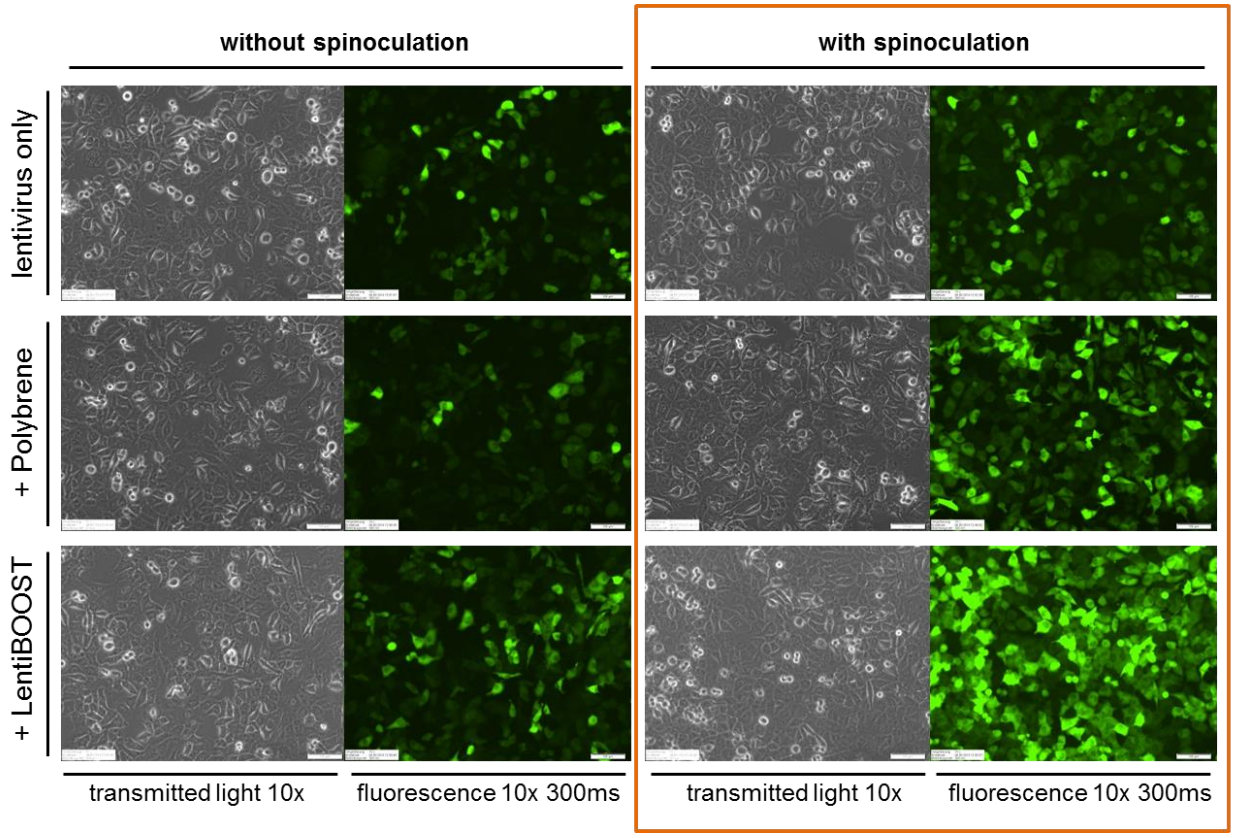

Fig. 1: Lentiviral transduction efficiencies in H1299 48h after transduction with lentivirus LV-CMV-GFP. Protocols according to the instructions of this manual

MATERIALS SUPPLIED

LentiBOOST™:

SB-P-LV-101-01 1 vial à 500 µl LentiBOOST **100x Solution A** (for 100 transductions in 24well)
1 vial à 500 µl LentiBOOST **100x Solution B** (for 100 transductions in 24well)

SB-P-LV-101-02 1 vial à 1500 µl LentiBOOST **100x Solution A** (for 300 transductions in 24well)
1 vial à 1500 µl LentiBOOST **100x Solution B** (for 300 transductions in 24well)

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 and 30 for transduction and to add LentiBOOST™ to the standard concentration of 1:100 (solution A and B each) of the total volume (medium+virus). LentiBOOST™ should be titrated 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.

- *Note: For cells (e.g. primary hematopoietic cells) which are sensitive to other transduction enhancers (e.g. Polybrene) it is recommended to use only solution B*

- Calculate the volume of lentivirus needed (see Table 1)
- Thaw lentivirus at 4 °C
- Add the appropriate amount of LentiBOOST™ Solution A and B 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

					Amount of infectious lentiviral particles					
Plate	**Cell number/well**	**Total Volume (Medium+Virus)**	**Volume LentiBOOST Solution A**	**Volume LentiBOOST Solution B**	**MOI 30**	**MOI 15**	**MOI 10**	**MOI 5**	**MOI 2**	**MOI 0**
6 well	1,00E+05	2 ml	20 µl	20 µl	3,0E+06	1,50E+06	1,0E+06	5,0E+05	2,0E+05	0

Table 1: Recommended volumes of medium and LentiBOOST™ to be used for lentiviral transductions in an initial experiment. For cells which are sensitive to other transduction enhancers (e.g. Polybrene) solution B can be used separately. It is recommended to titrate LentiBOOST™ in a second experiment in order to determine the minimal active concentration. Therefore, we recommend to dilute LentiBOOST Solution A and B 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 cell culture 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). A selection protocol for setting up a killing curve for Puromycin and G418 is provided in Appendix A: 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:

Problem	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 Solution A and B up to 1:20
		Try various combinations of Solution A and B with high MOI (e.g. MOI 20)
Low viability	Cells are sensitive to one component of LentiBOOST™	Try one well with Solution B only
		Try various combinations of Solution A and B with low MOI (e.g. MOI)
	Cells are sensitive to spinoculation	Try protocol without spinoculation Reduce duration Reduce velocity
	Cells are sensitive to lentiviral vectors	Change medium 4h after transduction or directly after centrifugation

APPENDIX A: Killing Curve

1. Seeding cells

The day before performing a killing curve, seed equal amounts of cells into cell culture plates. The number of cells to be plated per well depends on cell type. 1,5E+04 cells per 24 well were found to be suitable for most adherent growing cells, e.g. NIH-3T3.

2. Killing curve (24-well)

- At the day the killing curve is started, cells should exhibit ~30-50% confluence and good viability
- Prepare serial dilution according to Table 2
- Aspirate medium from cells and replaced it by 250 µl of the appropriate antibiotics dilution
- Incubate cells at normal growth conditions
- Optional: replace medium every 2-3 days
- Analyse cells after 5 days: Select the lowest concentration with no viable cells for further experiments

	Puromycin	
	Final concentration	stock solution **10mg/ml => dilute 1:10**
P10	8,000 µg/ml	16 µl in 2 ml medium
P9	5,000 µg/ml	serial dilution: 600 µl medium + 1000 µl previous dilution
P8	3,125 µg/ml	
P7	1,953 µg/ml	
P6	1,221 µg/ml	
P5	0,763 µg/ml	
P4	0,477 µg/ml	
P3	0,298 µg/ml	
P2	0,186 µg/ml	
P1	0,116 µg/ml	

	G418	
	Final concentration	stock solution **50,0 mg/ml**
G8	3.000 µg/ml	90 µl in 1,5 ml medium
G7	1.875 µg/ml	serial dilution: 600µl medium + 1000µl previous dilution
G6	1.172 µg/ml	
G5	732 µg/ml	
G4	458 µg/ml	
G3	286 µg/ml	
G2	179 µg/ml	
G1	112 µg/ml	

Table 2: Serial dilutions of antibiotic stock solution for setting up optimal selection conditions for stable cell line generation

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