Protocol

LentiBOOST™ Transduction Enhancer, Pharma Grade

Storage : -15 °C to -25 °C

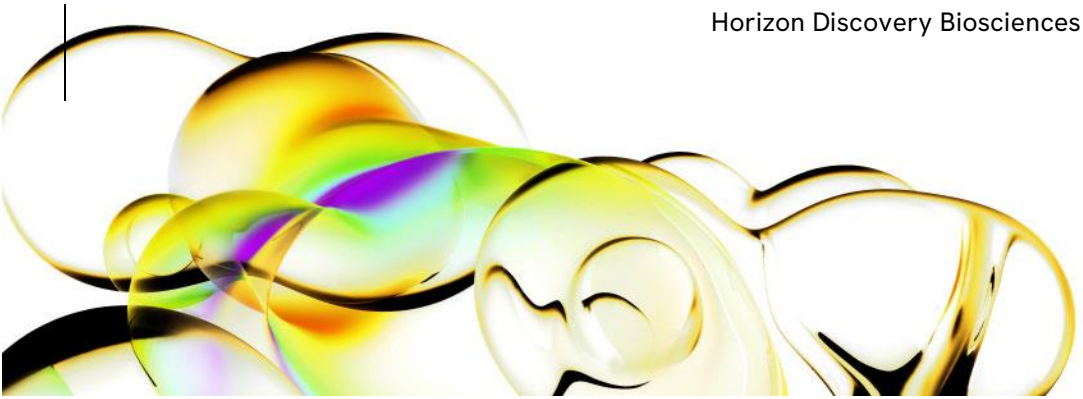
Shipped at room temperature

Version: 0001

July 2025

For research only. Not for use in diagnostic procedures.

Horizon Discovery Biosciences Ltd. (A Revvity Company)



Product Description

LentiBOOST™ Transduction Enhancer (TE) is designed to improve transduction efficiency and to reduce the quantity of virus required for effective gene transfer in *ex vivo* cell therapy applications. The reagent facilitates enhanced lentivirus-cell membrane fusion, leading to improved lentiviral copy number per cell without compromising cell viability or differentiation potential (Hauber I, et al, 2018). This means that regardless of whether the target lentiviral copy number per cell is single or multiple, a lower viral input will be sufficient to achieve the intended result. LentiBOOST TE has been validated across multiple cell types including primary T cells and $CD34^+$ hematopoietic stem cells (HSCs) and has been integrated into numerous clinical research protocols.

For more comprehensive guidance on optimizing lentiviral transduction, determining the multiplicity of infection (MOI), and other product-specific protocols, please refer to the extended technical manuals found on our website:

- SMARTvector Lentiviral shRNA Technical manual: https://horizondiscovery.com/-/media/Files/Horizon/resources/Technical-manuals/smartvector-constitutive-manual.pdf?sc_lang=en)
- Edit-R CRISPR-Cas9 Gene Engineering with All-in-one lentiviral Cas9 and sgRNA Technical manual: https://horizondiscovery.com/-/media/Files/Horizon/resources/Technical-manuals/edit-r-aio-lentiviral-sgrna-manual.pdf?sc_lang=en)
- Trans-Lentiviral Packaging Kit Technical Manual: https://horizondiscovery.com/-/media/Files/Horizon/resources/Technical-manuals/trans-lentiviral-packaging-manual.pdf?sc_lang=en

Lentiviral transduction protocol with using LentiBOOST TE

It is recommended to thaw LentiBOOST TE at a temperature between 4 °C and 25 °C. Before opening the tube, briefly centrifuge to collect any liquid from the lid. For long-term storage, LentiBOOST TE can be aliquoted using aseptic technique and stored at -15 °C and -25 °C. Alternatively, LentiBOOST TE can be stored at 4 °C for up to 1 month. No loss of functionality* has been observed after up to 5 freeze-thaw cycles.

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

PLEASE NOTE; the protocol given below contains suggestions based on internal data. Protocols must be adapted depending on experiment-specific conditions.

Day 1:

1. Seed cells in Growth Medium. The cell number should be extrapolated from the number of cells desired at the time of transduction and the doubling time of your cell type. Incubate overnight.

Note: Growth Medium: antibiotic-free cell culture medium (with serum and/or supplements) recommended for maintenance of the cells.

Day 2:

1. The next day, remove the medium and add optimized Transduction Medium with the appropriate amount of lentiviral particles and LentiBOOST TE so that the cells are just covered.

Note: When possible, the transduction of cells with lentiviral particles should be performed in a small volume of low-serum (0.5-2%) or serum-free medium. For cells sensitive to low serum conditions, transduction optimization can be performed in complete medium.

For optimal application, LentiBOOST TE is typically diluted between 1:100 to 1:400. It is recommended to start with 1 mg/ml (1:100) of the total volume or can be titrated in a range of 5 mg/ml – 0.1 mg/ml (1:20-1:1000) to determine the minimal active concentration.

Transduction duration can vary between 4-24 hours and will depend on the cells of interest.

2. After the appropriate transduction time, replace the Transduction Medium with Growth Medium and incubate cells for 48-72 hours.

Days 4-15 (for antibiotic selection):

Use the appropriate concentration of blasticidin or puromycin and the minimum number of days required to kill non-transduced cells as determined by an antibiotic dose response curve.

1. At 48-72 hours post-transduction, begin blasticidin or puromycin selection to remove non-transduced cells.
2. Every 48-72 hours replace with fresh Selection Medium containing blasticidin or puromycin and passage cells as needed.

Once an enriched cell population has been obtained (7-15 days), expand the cell population to generate sufficient cells for your experiment and/or prepare frozen aliquots for future use. If desired, proceed with isolating clonal cell lines.

Days 4-15 (for fluorescent selection):

1. At 24-48 post-transduction, check the cells for expression of EGFP. Cells can be subjected to enrichment by FACS or studied individually.
2. If cells have been subjected to enrichment, expand the enriched cell population to generate sufficient cells for your experiment and/or prepare frozen aliquots for future use. If desired, proceed with isolating clonal cell lines.

References

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

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If you have any questions contact
t +44 (0) 1223 976 000 (UK) or +1 800 235 9880 (USA); +1 303 604 9499 (USA)
f +44 (0)1223 655 581
w https://horizondiscovery.com/en/contact-us
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom
For more information
To find the contact information in your country for your technology of interest, please visit us at horizondiscovery.com/contact-us
Horizon Discovery, 8100 Cambridge Research Park, Waterbeach, Cambridge, CB25 9TL, United Kingdom

LentiBOOST™ is manufactured by Revvity Gene Dlivery GmbH, Am Haag 6, 82166 Gräfelfing, Germany

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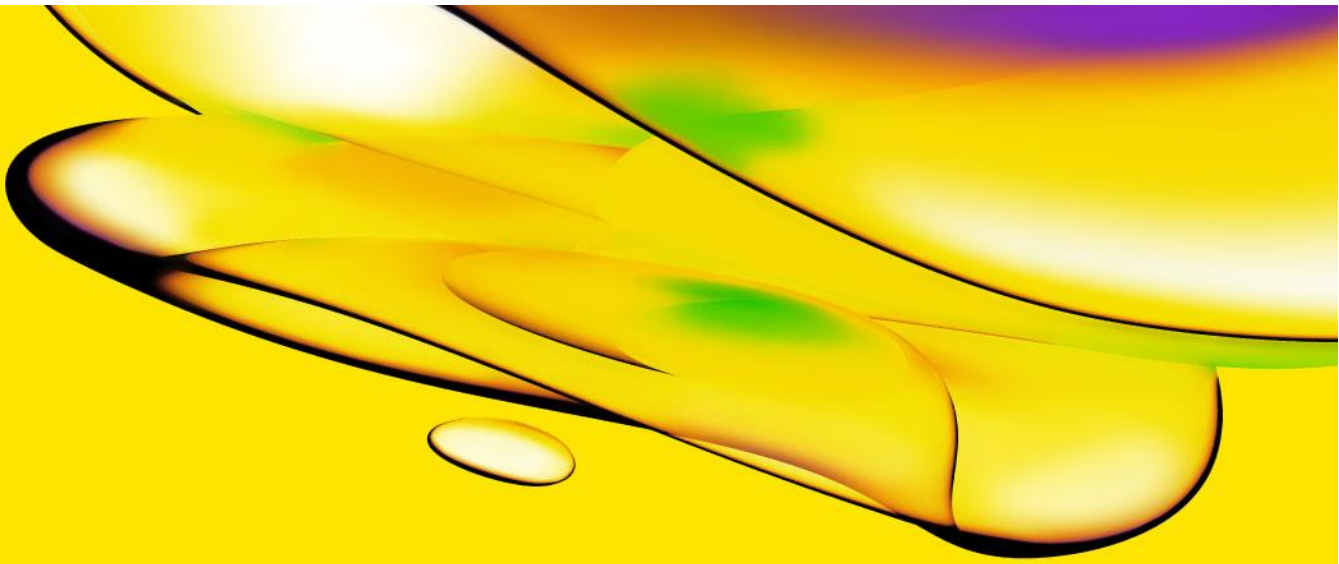

Horizon Discovery Biosciences Ltd. (A Revvity Company)
8100 Cambridge Research Park
Waterbeach, Cambridge, CB25 9TL, United Kingdom

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