



Pre-made Lentivirus for Target Overexpression

CAT#	Product	Accession ID
LVP1511	hPD1-mCherry Fusion (Bsd) Lentivirus	NM_000345

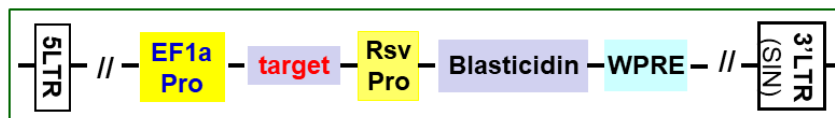
Amount: 200ul/vial (1 x 10⁸ IFU/ml)/each. (Premixed with Polybrene, 10x / 60 ug/ml)

Storage: < -70 °C, avoid repeat freeze/thaw cycles. Stable for 6 months at < -70oC.

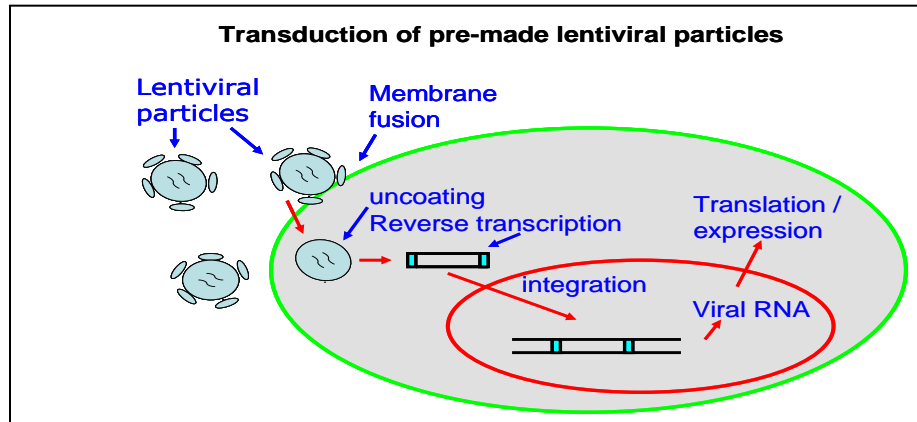
Product Description:

GenTarget's Lentivector system is Human Immunodeficiency Virus-1 (HIV) based lentivector plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into most mammalian cell types, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making lentivirus a great gene transfer agent.

This premade Lentivirus is generated from GenTarget's 3rd generation lentivector. It adapted the most advanced biosafety features, including the self-inactivation feature in its 3' LTR, and only generates the replication-incompetent lentivirus.



This lentivirus express a **human PD1 gene fused with mCherry report (hPD1-mCherry Fusion)**, under enhanced **EF1a** promoter. (see lentivector's scheme above). The lentivirus contains the Blastidicin antibiotic selection marker (**Bsd**), which allows the selection of the transduced cells by Fluorescent signal, and via antibiotic killing selection. The fluorescent signal provides a convenient, real-time means to monitor the particles' performance.



Key features:

- 1) High target expression levels driven by enhanced EF1a promoter;
- 2) Easy transduction monitoring by the fluorescent signal;
- 3) Dual selection markers;
- 4) Ready to use and easy to use: simply add it into your cell culture, No need any other reagents;

Transduction Protocols:

1. Transduction Protocol for Adhesive cells:

Note: Pre-made lentivirus is provided ready to use, so it can be simply added into your cell culture; the amount of virus to add depends on cell type. For quick transduction, add 50 μ l of virus into each well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

Day 0:

Seed cells in complete medium at the appropriate density and incubate overnight.

Note: at the time of transduction, cells should be 50%-75% confluent. For example, seed HeLa cells at 0.5×10^5 /ml x 0.5ml in a well of a 24-well plate.

Day 1:

- Thaw the pre-made lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI.



- Return cells to 37°C, CO₂ incubator. Do nothing.
Note: Try to avoid freezing and thawing. If you do not use all of the virus at one time, you may re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At 48hr~72hr (Depend upon cell type) after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

Day 3 + (optional):

Sort transduced cells by FACS, or select by antibiotic killing. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

2. Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO₂ incubator if necessary.

Measure cell density (not grow over 3 million/ml), measured viability should be > 90%. Dilute cells into 1 x 10⁶ cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO₂ incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium. Continue growing cells in CO₂ incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence



positive cells and maintain antibiotic selection to generate a stable cell line.

Note: Filter wavelength settings for mCherry: 587nm/60nm (Ex/Em)

Safety Precaution:

Gentarget lentiviral particles adapts must advanced lentiviral safety features (using the third generation vectors with self-inactivation SIN-3UTR), and the premade lentivirus is replication incompetent. However, please use extra caution when using lentiviral particles. Use the lentiviral particles in Biosafety II cabinet. Wear glove all the time when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. J Virol. 2000 November; 74(22): 10778–10784.
2. Hum Gene Ther (2003) 14: 1089-105.
3. Mol Ther (2002) 6: 162-8.
4. NIH Guidelines for [Biosafety Considerations for Research with Lentiviral Vectors](#). (Link).

Warranty:

This product is for research use only. It is warranted to meet its quality as described when used in accordance with its instructions. GenTarget disclaims any implied warranty of this product for particular application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with the products. GenTarget's sole remedy for breach of this warranty should be, at GenTarget's option, to replace the products.

Attachment: GenTarget's Pre-made lentivirus Products:

Product Category	Product Description (please click category name to see product's pages)
Target ExpressionUOT	Premade lentivirus expressin a human, mouse or rat gene with RFP-Blastididin fusion dual markers.
Pathway Reporter	Different Report lentivirus (Luc, RFP, GFP, SEAP) under a Ppathway specific response promoter
Cell Immortalization	Comprehesive lentivirus for cell immortalization, for different cell types.
Cell Specific reporter	Different Report lentivirus driven by cell specific promoter.



Viral Antigens	Lentivirus express a specific viral antigen
Viral Like Particle (VLP)	Lentiviral particles pseudo-typed with high density of surface envelope protein.
Immuno Therapy	Lentivirus products for Immuno Therapy application.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, GFP / RFP / CFP / BFP / YFP .
Luciferase expressionUOT	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla with different antibiotic selection markers.
CRE recombinaseUOT	Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic markers.
LoxP ColorSwitchUOT	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Preamde lentivirus express humanized wild-type Cas9 endonuclease for genomic editing with CRISPR
TetR inducible expression repressorUOT	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factorsUOT	Premde lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibiotic markers
T-antigen Expression	Express SV40 large T antigen with different selection markers
Cell Organelle imagingUOT	Premade lentivirus for cell organelle imaging. The fluorescent marker GFP/RFP/CFP was sub-cellular localized in different cell organelle for living cell imaging.
LacZ expression	Express different full length β- galactosidase (lacZ) with different selection markers
Anti-miNA lentivirusUOT	Pre-made lentivirus expression a specific anti-miRNA cassette.
Fluorescent-ORF fusionUOT	Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target.
Pre-made shRNA	Premade shRNA lentivirus for knockdown a specific genes (P53, LacZ, Luciferase and more).



lentivirusUOT	
microRNA and anti-microRNA lentivirusUOT	Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA.
Negative control lentivirusesUOT	Premade negative control lentivirus with different markers : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing specific enzymes with different selection markers.