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Pre-made Target Over-expression Lentivirus

CAT#	Product	Accession ID
LVPxxx	Concentrated Expression Lentivirus (200ul at titer of 1x 10^8 IFU/ml)	

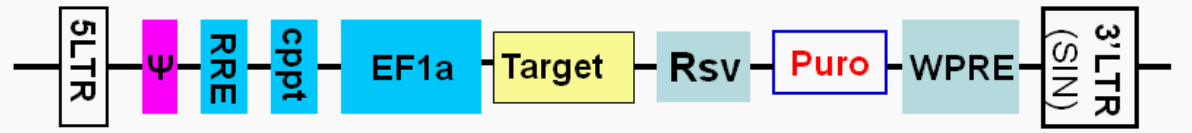
Amount: 200ul/vial (1 x 10^8 IFU/ml)/each.
Storage: <-70 °C, avoid repeat freeze/thaw cycles. Stable for 6 months at <-70oC.

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 target over-expression Lentivirus products, is generated from GenTarget's Lentivector. The target is expressed under the enhance EF1a promoter which is less cell type dependent and not be silenced *In vitro* and *In vivo*, also strong in many primary cells and T cells, B cells, and more. The lentivirus contains the **Puromycin** selection under RSV promoter.

The Lentivector is adapted the most advanced biosafety features, including the self-inactivation feature in its 3' LTR, and only generates the replication-incompetent lentivirus. See the lentivector's map scheme below.



The Negative Control Lentivirus can be used to establish the controls for lentivirus treatment in a given cell line. The control lentivirus, CAT# *EF1a-Null-Puro*, has the identical lentivector backbone with the puromycin resistance, as that of target expression, but does not express any target. Please see also "*FAQs about premade lentiviral particles*".

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Key features:

1) High target expression levels driven by strong enhanced EF1a promoter, best for primary cells, suspension cells, T cells, B cells and more;
2) Puromycin killing selection;
3) Ready to use and easy to use: simply add it into your cell culture, No need any other reagents or procedures;

Transduction Protocols:

1) Transduction Protocol for Adhesive cells :

Note: lentivirus is provided as ready to use. Simply add it into your cell culture; the amount to add depends on cell type.
Quick transduction protocol: add 50 µl of virus into each well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), check transduction rate by fluorescence signal. For stable cell line generation, pass cells into medium containing antibiotic or carry out the fluorescence cell sorting.

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 x 10^5/ml x 0.5ml in a well of a 24-well plate.

Day 1:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- Thaw the lentiviral stock at room temperature and add the appropriate amount of virus stock to obtain the desired MOI, normally use MOI from 2 to 10.
- Return cells to 37°C, CO_2 incubator.
  Note: If you do not use all of the virus at one time, you can re-freeze the virus at -80 °C for future use; virus titer will decrease by ~10% for each freeze/thaw cycle.

Day 3:

At ~72hr after transduction, check the transduction rate under fluorescence microscopy or calculate the exact transduction rate by

flow cytometry (FACS or Guava). Carry out the expression detection assay.

Day 3 +

Sort transduced cells by FACS, or select the transduced cell by antibiotic resistance. 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 CO^2 incubator if necessary.

Measure cell density. When density has reached ~3 x 10^6 cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10^6 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 containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO2 incubator.

Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

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 Bio-safety II cabinet. Wear glove all the time when handling Lentiviral particles!

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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)
Human, mouse or rat ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Pathway Reporter	Premade lentivirus reporter express a luminescence or fluorescent report (firefly **Luciferase**, **Renilla** luciferase, **RFP** or **GFP** fluorescent marker) under a pathway specific promoter.
Cell-Specific Reporter	Premade lentivirus reporter for targeting expression of a luminescence or fluorescent report (firefly **Luciferase**, **Renilla** luciferase, **RFP** or **GFP** fluorescent marker) under a cell type specific promoter.
Cell Immortalization	Premade different set lentivirus for primary cell immortalization, including **SV40 larget T antigen**, human **TER**T, siRNA-P53, EBV genes, HpV16 E6, Adenovial E1A, HOSA9, CDK4 cMyc KRas and more
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.

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LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing with **CRISPR**
TetR inducible expression repressor	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
Cell Organelle imaging	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 lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
Pre-made shRNA lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Negative control lentiviruses	Premade **negative control lentivirus with different markers**: serves as the negative control of lentivurs treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme expression	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.