



Expression Lentivirus for SV40 large T-antigen

| CAT# | Product Name | Amounts |
|------------------------------|---|---|
| LVP016-Neo | SV40 Large T-antigen (CMV, Neo) lentivirus | 200ul, (1 x 10 ⁷ IFU/mL) in DMEM medium, premixed with Polybrene |
| LVP016-Bsd | SV40 Large T-antigen (CMV, Bsd) lentivirus | |
| LVP016-Puro | SV40 Large T-antigen (CMV, Puro) lentivirus | |
| LVP016-Zeo | SV40 Large T-antigen (CMV, Zeo) lentivirus | |
| LVP016-Hygro | SV40 Large T-antigen (CMV, Hygro) lentivirus | |
| LVP016-GB | SV40 Large T-antigen (CMV, GFP-Bsd) lentivirus | |
| LVP016-GP | SV40 Large T-antigen (CMV, GFP-Puro) lentivirus | |
| LVP016-RB | SV40 Large T-antigen (CMV, RFP-Bsd) lentivirus | |
| LVP016-RP | SV40 Large T-antigen (CMV, RFP-Puro) lentivirus | |
| LVP016 | SV40 Large T-antigen (CMV, no selection) lentivirus | |
| LVP557-Neo | SV40 Large T-antigen (EF1a, Neo) lentivirus | |
| LVP557-Bsd | SV40 Large T-antigen (EF1a, Bsd) lentivirus | |
| LVP557-Puro | SV40 Large T-antigen (EF1a, Puro) lentivirus | |
| LVP557-Zeo | SV40 Large T-antigen (EF1a, Zeo) lentivirus | |
| LVP557-Hygro | SV40 Large T-antigen (EF1a, Hygro) lentivirus | |
| LVP557-GB | SV40 Large T-antigen (EF1a, GFP-Bsd) lentivirus | |
| LVP557-GP | SV40 Large T-antigen (EF1a, GFP-Puro) lentivirus | |
| LVP557-RB | SV40 Large T-antigen (EF1a, RFP-Bsd) lentivirus | |
| LVP557-RP | SV40 Large T-antigen (EF1a, RFP-Puro) lentivirus | |



| | | |
|---|--|---|
| <u>LVP557</u> | SV40 Large T-antigen (EF1a, no selection) lentivirus | |
| <u>LVP016-Neo-PBS</u> | SV40 Large T-antigen (CMV, Neo) lentivirus in PBS | 200ul, (1 x 10 ⁸ IFU/mL) in PBS solution, premixed with Polybrene |
| <u>LVP016-Bsd-PBS</u> | SV40 Large T-antigen (CMV, Bsd) lentivirus in PBS | |
| <u>LVP016-Puro-PBS</u> | SV40 Large T-antigen (CMV, Puro) lentivirus in PBS | |
| <u>LVP016-Zeo-PBS</u> | SV40 Large T-antigen (CMV, Zeo) lentivirus in PBS | |
| <u>LVP016-Hygro-PBS</u> | SV40 Large T-antigen (CMV, Hygro) lentivirus in PBS | |
| <u>LVP016-GB-PBS</u> | SV40 Large T-antigen (CMV, GFP-Bsd) lentivirus in PBS | |
| <u>LVP016-GP-PBS</u> | SV40 Large T-antigen (CMV, GFP-Puro) lentivirus in PBS | |
| <u>LVP016-RB-PBS</u> | SV40 Large T-antigen (CMV, RFP-Bsd) lentivirus in PBS | |
| <u>LVP016-RP-PBS</u> | SV40 Large T-antigen (CMV, RFP-Puro) lentivirus in PBS | |
| <u>LVP016-PBS</u> | SV40 Large T-antigen (CMV, no selection) lentivirus in PBS | |
| <u>LVP557-Neo-PBS</u> | SV40 Large T-antigen (EF1a, Neo) lentivirus in PBS | |
| <u>LVP557-Bsd-PBS</u> | SV40 Large T-antigen (EF1a, Bsd) lentivirus in PBS | |
| <u>LVP557-Puro-PBS</u> | SV40 Large T-antigen (EF1a, Puro) lentivirus in PBS | |
| <u>LVP557-Zeo-PBS</u> | SV40 Large T-antigen (EF1a, Zeo) lentivirus in PBS | |
| <u>LVP557-Hygro-PBS</u> | SV40 Large T-antigen (EF1a, Hygro) lentivirus in PBS | |
| <u>LVP557-GB-PBS</u> | SV40 Large T-antigen (EF1a, GFP-Bsd) lentivirus in PBS | |
| <u>LVP557-GP-PBS</u> | SV40 Large T-antigen (EF1a, GFP-Puro) lentivirus in PBS | |
| <u>LVP557-RB-PBS</u> | SV40 Large T-antigen (EF1a, RFP-Bsd) lentivirus in PBS | |
| <u>LVP557-RP-PBS</u> | SV40 Large T-antigen (EF1a, RFP-Puro) lentivirus in PBS | |
| <u>LVP557-PBS</u> | SV40 Large T-antigen (EF1a, no selection) lentivirus in PBS | |



Storage: <-70 °C, avoid repeat freeze/thaw cycles, stable for > 6 months.

Product Description:

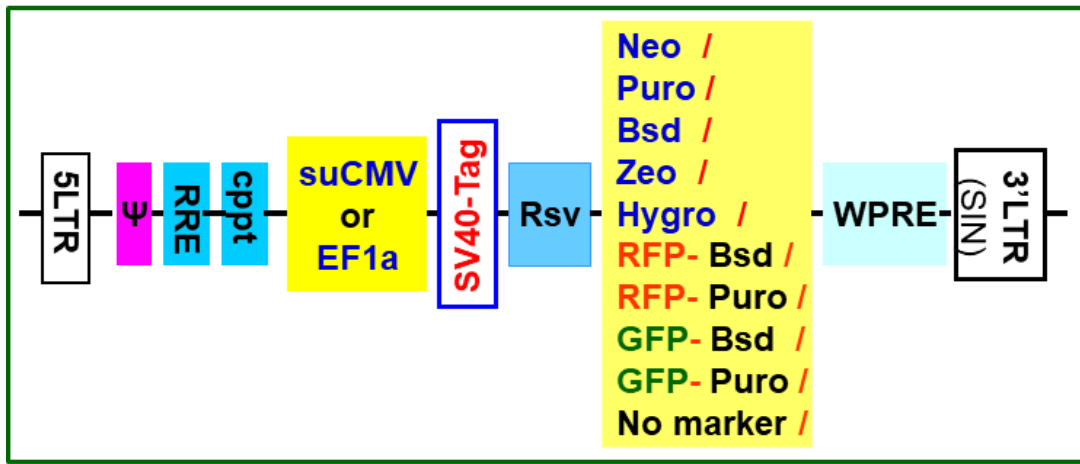
Lentiviral particles or lentivirus is a gene delivery tool produced from lentivectors for gene expression or knockdown. GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) that can be transduced into almost all kinds of mammalian cells, 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 it a great gene transfer agent.

SV40 large T antigen (Simian Vacuolating Virus 40 T-Antigen) (click to see its coding sequence), is a hexamer protein involved in viral genome replication and regulation of the host cell cycle. It is used as a model protein to study nuclear localization signals and other viral applications. **It also widely used for cell immortalization for variety of cell types.**

SV40 large T-antigen expression lentivirus are generated from GenTarget's re-engineered lentivector system. The SV40 large T-antigen gene, was fully verified by sequencing, expressed under an enhanced **CMV** (suCMV) or enhanced **EF1a** promoter. VSV-G pseudotyped lentiviral particles are generated in 293T cell, and packaged in DMEM medium with 10% serum or in serum free PBS solution, supplied as 200ul/per vial at $1\sim5 \times 10^7$ IFU/ml.

T-antigen was expression under either **suCMV promoter** (which demonstrate the strongest activity in most cell types) an **enhanced EF1a promoter** (which is active in almost all cell types and less likely to be silenced during long-term culture).

Each Lentivirus is featured with a selection marker (**Neomycin, Puromycin, Blasticidin, Zeocin, Hygromycin**), or an antibiotic-fluorescent fusion dual maker (**RFP-Bsd, RFP-Puro, GFP-Bsd, GFP-Puro**). (see **vector map scheme** above). When desired, you can select the lentivirus does not contain any selection markers. See the scheme below showing the lentivector's core structure.



The products are provided as 200 ul aliquots in two formats:

- 1) in DMEM medium containing 10% and 10x Polybrene (60 ug/ml);
- 2) in PBS solution for usage in serum-free cell culture;

For general questions about our ready-to-use particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf) on our website.
(<http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf>).

Key features:

1. **Flexible selection markers:** you can select the product with your desired antibiotic selection marker, or fluorescent selection marker, or no any selection.
2. The strongest [suCMV promoter](#) make the pre-made virus a ideal tool for mammalian protein expression, stable cell line construction and enzymatic assays both in vivo or in vitro (see schematic vector map below).
3. The enhance **EF1a promoter** is active in all cell types and do not be silenced during long-term culture.
4. **Each and ready to use:** Simply add 50ul into one well culture in 24-well plate.

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 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), 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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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 1×10^6 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 μ l 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. 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:

| | |
|------------------------|---------|
| BFP filter: ~Ex380 | ~Em460; |
| GFP filter: ~Ex450-490 | ~Em525; |
| RFP filter: ~Ex558 | ~Em583; |

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! 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 specific 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 product categories.

| Product Category | Product Description (please click into each category's page) |
|---|--|
| Pathway Reporter | Repoter Lentivirus for all kinds of pathway screening assays |
| Cell Immortalization | Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al. |
| ImmunoOncology Research | Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus; |
| CAR-T, TCR Lentivirus | CARs Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; TCRs : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6; |
| CRISPR Gene Editing | Preamde lentivirus express humanized wild-type Cas9 endonuclease, the dCas9 , gRNAs, CRISPR gene editing research |
| Epigenomic: CRISPRi and CRISPRa | " dCas9-Protein " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa). |
| Cell-Specific Reporter | a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter |
| Infectious Antigens | Lentivirus that express all kinds of infectious antigens with C-term 6His-tag. |
| Virus Like Particles (VLP) | Lentiviral Like Particles, pseudo-typed with a different envelope proteins. |
| Non-integrating LV | Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis. |



| Product Category | Product Description (please click into each category's page) |
|--|---|
| shRNA Knockdown | Knockdown verified and customized shRNA lentivirus for target knockdown, |
| microRNA lentivirus | Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA. |
| Anti-miRNA lentivirus | Pre-made lentivirus expression a specific anti-miRNA cassette. |
| Human and mouse ORFs | Premade lentivirus expressing a human, mouse or rat gene with RFP-Blasticidin fusion dual markers. |
| Luciferase expression | Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla, Red-Luc and more , with different antibiotic selection markers. |
| Fluorescent Markers | Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, mRFP, unstable GFP and others. |
| Luminescent Imaging | Lentivirus express Nano-Lantern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms |
| Sub-cellular Imaging | Lentivirus contain a well-defined organelle targeting signal fused to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways. |
| Cytoskeleton Imaging | A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure |
| Unstable GFP | Lentivirus express the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection |
| near-infrared RFP | The near-infrared Red fluorescent (mRFP) expression Lentivirus provides the whole-body images with better contrast and brighter images |
| Fluorescent-ORF fusion | Pre-made lentivirus expression a " GFP/RFP/CFP-ORF " fusion target. |
| CRE recombinase | Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic markers. |



| Product Category | Product Description (please click into each category's page) |
|---|---|
| CRE, Flp ColorSwitch | Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo. |
| SEAP Reporter | lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter), |
| TetR Repressor | Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system. |
| rtTA Expression | rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF inducible system. |
| iPS factors | Premde lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLF4) factors with different fluorescent and antibitoic markers |
| LacZ expression | Express different full length β- galactosidase (lacZ) with different selection markers |
| 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 a specific enzymes with different selection markers. |
| Ultra titer lentivirus | Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells. |