



Pre-made Expression Lentivirus for SV40 large T-antigen

CAT#	Product Name	Amounts
<u>LVP016-Neo</u>	SV40 Large T-antigen (CMV, Neo) lentivirus	200ul, (1 x 10 ⁷ IFU/mL) in DMEM medium containing 10% FBS and 10 x (60 ug/m) polybrene
<u>LVP016-Bsd</u>	SV40 Large T-antigen (CMV, Bsd) lentivirus	
<u>LVP016-Puro</u>	SV40 Large T-antigen (CMV, Puro) lentivirus	
<u>LVP016-GB</u>	SV40 Large T-antigen (CMV, GFP-Bsd) lentivirus	
<u>LVP016-GP</u>	SV40 Large T-antigen (CMV, GFP-Puro) lentivirus	
<u>LVP016-RB</u>	SV40 Large T-antigen (CMV, RFP-Bsd) lentivirus	
<u>LVP016-RP</u>	SV40 Large T-antigen (CMV, RFP-Puro) lentivirus	
<u>LVP557-Neo</u>	SV40 Large T-antigen (EF1a, Neo) lentivirus	
<u>LVP557-Bsd</u>	SV40 Large T-antigen (EF1a, Bsd) lentivirus	
<u>LVP557-Puro</u>	SV40 Large T-antigen (EF1a, Puro) lentivirus	
<u>LVP557-GB</u>	SV40 Large T-antigen (EF1a, GFP-Bsd) lentivirus	
<u>LVP557-GP</u>	SV40 Large T-antigen (EF1a, GFP-Puro) lentivirus	
<u>LVP557-RB</u>	SV40 Large T-antigen (EF1a, RFP-Bsd) lentivirus	
<u>LVP557-RP</u>	SV40 Large T-antigen (EF1a, RFP-Puro) lentivirus	
<u>LVP016-Neo-PBS</u>	SV40 Large T-antigen (CMV, Neo) lentivirus in PBS	200ul, (5 x 10 ⁷ IFU/mL) in PBS solution
<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-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>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-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	

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.

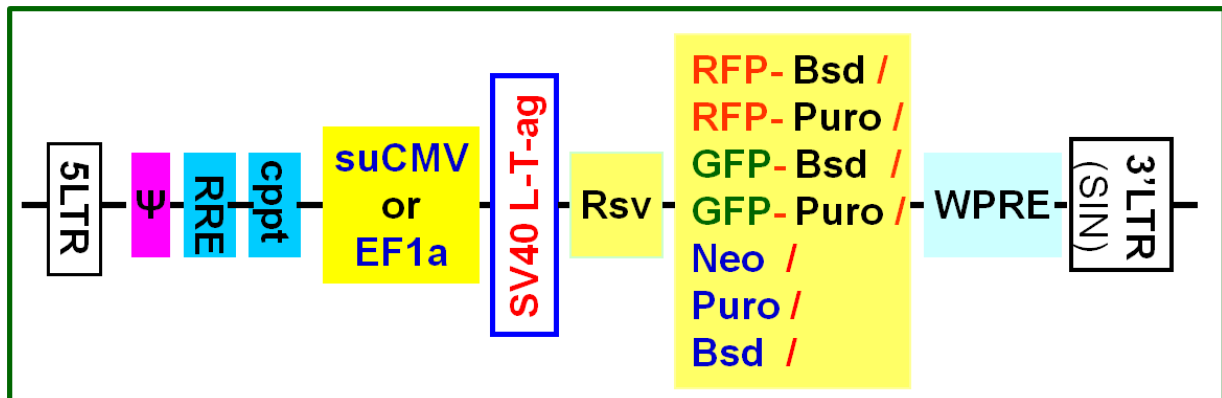
SV40 large T antigen (Simian Vacuolating Virus 40 T-Antigen) is a hexamer protein involved in viral genome replication and regulation of the host cell cycle. It is used as a model protein to study nuclear localization signals and other viral applications. **It also widely used for cell immortalization 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 $1\sim 5 \times 10^7$ 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 particle featured with a selection marker with or without a fluorescent maker as well. (see **vector map scheme** below).



T-ag lentivirus 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. Each lentiviral particles contain a specific resistant marker, used for selecting the transduced cells or generating stable cell lines by antibiotics selection or via fluorescent cell sorting.
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. **The lentivirus are ready and easy 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 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:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- 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.

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 ~72hr 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, and select for 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 CO² incubator if necessary.

Measure cell density. When density has reached $\sim 3 \times 10^6$ cells/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 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.

Note: Filter wavelength settings:

GFP filter: \sim Ex450-490; \sim Em525; **RFP** filter: \sim Ex545; \sim Em620;

Safety Precaution:

GenTarget lentiviral particles adapt 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 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.

Related Products: GenTarget's pre-made lentivirus product category.

Product Category	Product Description (please click category name to see product's pages)
Human, mouse or rat ORFs	Premade lentivirus expressing a human, mouse or rat gene with RFP-Blasticidin fusion dual markers.
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.
Signal Pathway Lentivirus	Lentivirus expressing a luminescence or fluorescent report (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a pathway specific promoter
Fluorescent markers	Premade lentivirus expressing 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 fluorescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Premade lentivirus expressing humanized wild-type Cas9 endonuclease for genomic editing with CRISPR
TetR inducible expression repressor	Premade lentivirus expressing TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factors	Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FLK4) factors with different fluorescent and antibiotic markers
T-antigen Expression	Express SV40 large T antigen with different selection markers
Cell Organelle	Premade lentivirus for cell organelle imaging. The fluorescent marker GFP/RFP/CFP was sub-cellular localized in different



imaging	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 lentiviruses 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.