

7930 Arjons Drive, Suite B San Diego, CA 92126, USA Phone: 1 (858) 265-6446 Fax: 1 (800) 380-4198

Email: orders@gentarget.com

SV40 large T-antigen Expression Lentivirus

CAT#	Product Name	Amounts
LVP016-Neo-PBS	SV40 Large T-antigen	
	(CMV, Neo) lentivirus in PBS	
LVP016-Bsd-PBS	SV40 Large T-antigen	
	(CMV, Bsd) lentivirus in PBS	
LVP016-Puro-PBS	SV40 Large T-antigen	
	(CMV, Puro) lentivirus in PBS	
LVP016-Zeo-PBS	SV40 Large T-antigen	
	(CMV, Zeo) lentivirus in PBS	
LVP016-Hygro-PBS	SV40 Large T-antigen	
	(CMV, Hygro) lentivirus in PBS	_
LVP016-GB-PBS	SV40 Large T-antigen	
	(CMV, GFP-Bsd) lentivirus in PBS	
LVP016-GP-PBS	SV40 Large T-antigen	
	(CMV, GFP-Puro) lentivirus in PBS	
LVP016-RB-PBS	SV40 Large T-antigen	
	(CMV, RFP-Bsd) lentivirus in PBS	200ul, (1 x 10 ⁸
LVP016-RP-PBS	SV40 Large T-antigen (CMV, RFP-Puro) lentivirus in PBS	
	SV40 Large T-antigen	
LVP016-PBS	(CMV, no selection) lentivirus in PBS	
	SV40 Large T-antigen	IFU/mL)
LVP557-Neo-PBS	(EF1a, Neo) lentivirus in PBS	in PBS solution, premixed with Polybrene
	SV40 Large T-antigen	
LVP557-Bsd-PBS	(EF1a, Bsd) lentivirus in PBS	
LVDEEZ Davis DDC	SV40 Large T-antigen	
LVP557-Puro-PBS	(EF1a, Puro) lentivirus in PBS	
LVDEE7-700-DRS	SV40 Large T-antigen	
LVP557-Zeo-PBS	(EF1a, Zeo) lentivirus in PBS	
LVP557-Hygro-PBS	SV40 Large T-antigen	
LVI 337 TIYGIO I BS	(EF1a, Hygro) lentivirus in PBS	
LVP557-GB-PBS	SV40 Large T-antigen	
277 00 7 00	(EF1a, GFP-Bsd) lentivirus in PBS	
LVP557-GP-PBS	SV40 Large T-antigen	
	(EF1a, GFP-Puro) lentivirus in PBS	
LVP557-RB-PBS	SV40 Large T-antigen	
	(EF1a, RFP-Bsd) lentivirus in PBS	4
LVP557-RP-PBS	SV40 Large T-antigen	
	(EF1a, RFP-Puro) lentivirus in PBS	-
LVP557-PBS	SV40 Large T-antigen	



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(EF1a, no selection) lentivirus in PBS

Storage: -80 °C, avoid repeat freeze/thaw cycles, stable for > 6 months.

1. 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.

<u>SV40 large T antigen</u> (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.

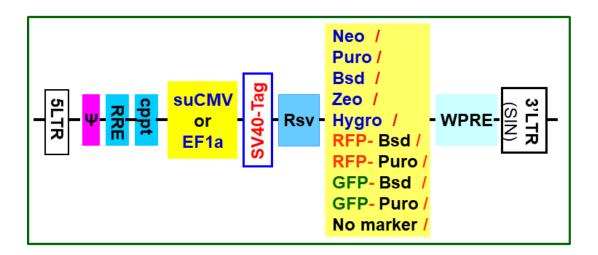
SV40 large T-antigen expression lentivirus are generated from GenTarget's re-engineered lentivector system. The SV40 large T-antigen gene, was fully verified by sequencing, expressed under an enhanced **CMV** (suCMV) or enhanced **EF1a** promoter. VSV-G pseudotyped lentiviral particles are generated 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**, or not contain any selection markers). See the scheme below showing the lentivector's core structure.



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The products are provided as 200 ul aliquots containing 10x Polybrene (60 ug/ml).

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/FAO-Premade-Lentiviral-particles.pdf).

2. 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 <u>suCMV promoter</u> make the product a highly effective cell-immortalization reagents.
- 3) The enhance **EF1a promoter** is active in all cell types and do not be silenced during long-term culture and best for some cell types.
- 4) **Each and ready to use:** Simply add 50ul into one well culture in 24-well plate.

3. Transduction Protocols:

Note: Pre-made lentivirus is provided ready to use status, simply added into your cell culture. The amount of lentivirus to add depends on cell type. In general, add 50 μ l of virus into one well in 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 if applicable. For stable cell line generation, pass cells into medium containing antibiotic or perform fluorescence cell sorting



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followed by antibiotic selection. (**Note**: for suspension cells or the "hard-to-transduced" cell type, you may need to double the virus amount added.)

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 $^{\circ}$ 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).

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525; RFP filter: ~Ex558 ~Em583;

4. 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.

5. References:



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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).

6. 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.

7. **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 humanzied 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



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Product	Product Description	
Category	(please click into each category's page)	
Infectious Antigens	Llentivirus 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.	
shRNA Knockdown	Knockdown verifeid 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-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.	
Human and mouse ORFs	Premade lentivirus expressin a human, mouse or rat gene with RFP-Blastididin fusion dual markers.	
<u>Luciferase</u> <u>expression</u>	Premade lentivirus for all kinds of luciferase protein expression: firefly and Renilla, Red-Luc and more, with different antibiotic selection markers.	
<u>Fluorescent</u> <u>Markers</u>	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.	
<u>Luminescent</u> <u>Imaging</u>	Lentivirus express Nano-Latern 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 fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of subcellular 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	
<u>Unstable GFP</u>	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection	



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Product	Product Description	
Category	(please click into each category's page)	
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression	
	Lentiviurs provides the whole-body images with better	
	contrast and brighter images	
Fluorescent-ORF	Pre-made lentivirus expression a "GFP/RFP/CFP-ORF"	
<u>fusion</u>	fusion target.	
	Premade lentivirus for expressing nuclear permeant	
CRE recombinase	CRE recombinase with different flurescent and antibiotic	
	markers.	
CRE, Flp	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or	
<u>ColorSwtich</u>	"FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the	
	CRE or Flp recombination event in vivo.	
CEAD Depositor	lentivirus expressing SEAP under different promoters	
SEAP Reporter	(TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal	
	pathway responsive promoter), Premade lentivirus expressin TetR (tetracycline	
TetR Repressor	regulator) protein, the repressor protein for the	
reux Kepressor	inducible expression system.	
	rtTA binds to the tetracycline operator element (TetO) in	
rtTA Expression	the presence of doxycycline (Dox). Used for Tet-On /OFF	
	inducible system.	
	Premde lentivirus for human and mouse iPS (Myc,	
iPS factors	NANOG, OCT4, SOX2, FLF4) factors with different	
	fluorescent and antibitoic markers	
LacZ expression	Express different full length β- galactosidase	
	(lacZ) with different selection markers	
	Premade negative control lentivirus with different	
Negative control	markers: serves as the negative control of lentivurs	
<u>lentiviruses</u>	treatment, for validation of the specificity of any	
Other Engume	lentivirus target expression effects.	
Other Enzyme	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.	
expression Ultra titer	Ultra-titer lentivirus used for the hard-to-transduced	
lentivirus	cells and for in vivo manipulation of sperm cells, or stem	
ichicivii us	cells.	
	CCIIOI	