

Pre-made unstable GFP Lentivirus

Catalog#	Product Name	Amounts
LVP1108-PBS	uGFP (TetCMV-Puro) Lentivirus	1x10 ⁸ IFU/ml x 200ul
LVP1109-PBS	uGFP (TetCMV-Bsd) Lentivirus	(provided in PBS solution, premixed with
LVP1110-PBS	uGFP (TetCMV-Neo) Lentivirus	10x Polybrene, 60ug/ml)

Note: The TetCMV promoter becomes a tetracycline inducible expression only when its repressor protein, TetR, is present. When TetR is absent, the TetCMV is a constitutive promoter. The TetR protein can be delivered by the premade <u>TetR Lentivirus</u>. The TetCMV driven expression of uGFP is first repressed by TetR, then induced by adding of tetracycline.

Storage: -80 °C, avoid repeat freeze/thaw cycles. Stable for 12 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.

The green fluorescent protein (GFP) is a widely used reporter, provide an easy detection in living cells. However, it is a very stable protein and accumulated in cells with long half-live, which limits its application that requires fast turnover responses in signal pathway assay and in knockdown / knockout detection. Therefore, the unstable GFP (uGFP) was created as the destabilized version reporter. The uGFP is best used for the time course induction and dose response kinetics, and for the fast response to knockdown or knockout.

A common used method to create uGFP is by fusing the GFP with a selfhydrolysis tag, destabilization tag or degradation domain. <u>Gentarget's uGFP</u> (click to see sequence) was made by fusing with a segment of mouse ornithine decarboxylase as the destabilize domain. It shows an *in vivo* half-life of about **2 hours**.



uGFP lentivirus were driven by <u>an optional inducible</u> CMV promoter (TetCMV). These lentivirus are provided with an antibiotic slection marker (Puromycin, Blasticidin or Neomycin). Please see the schematic lentivector core structure below.



*When TetR is absent, the TetCMV is a constitutive expression promoter without the need for any induction, which continuously express uGFP at high level. You will visualize GFP fluorescent signal at high level until the uGFP was knockdown or knockout.

Expression lentivectors were co-transfected with GenTarget's proprietary packaging mix (Cat# <u>HT-pack</u>) into 293T cells (cat# <u>TLV-C</u>). The pre-made lentiviral particles are VSV-G pseudotyped viruses. Each lot of virus is validated and quality is guaranteed.

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

GenTarget also provides lentiviral services for cloning your gene of interest and generates ready-to-use viral particles with the best prices and fastest turnaround time. Please see <u>our website</u> for details.

2. Transduction Protocols (How to use the product):

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 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 0.5×10^{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).

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;

Transduction Example:





Figure 1: uGFP Expression in Hela cells. Hela cells were transduced with 50ul of Premade uGFP lentivirus (#LVP1108) in 24-well plate. GFP signal was visualized at 72 hours after transduction (GFP filter: ~Ex450-490 ~Em525).

3. 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.

4. References:

J. B. C., Vol. 273, No. 52, Issue of December 25, pp. 34970-34975, 1998

5. 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.

6. Attachment: Genlarget's pre-made lentivirus product categ

Product Category	Product Description (please click into each category's page)	
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening assays	
<u>Cell</u> Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.	
<u>ImmunoOncology</u> <u>Research</u>	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;	
<u>CAR-T, TCR</u> <u>Lentivirus</u>	CARs Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; TCRs : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;	



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Product Category	Product Description (please click into each category's page)
<u>CRISPR Gene</u> <u>Editing</u>	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).
<u>Cell-Specific</u> <u>Reporter</u>	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	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
<u>Virus Like</u> Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
<u>Non-integrating</u> LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
<u>shRNA</u> <u>Knockdown</u>	Knockdown verifeid and customized shRNA lentivirus for target knockdown,
<u>microRNA</u> <u>lentivirus</u>	Premade lentivirus expression human or mouse precursor miRNA . And anti-miRNA lentivector and virus for human and mouse miRNA.
<u>Anti-miNA</u> <u>lentivirus</u>	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.
Luciferase expression	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.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms



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Product	Product Description	
Category	(please click into each category's page)	
Sub-cellular	Lentivirus contain a well-defined organelle targeting	
<u>Imaging</u>	signal fusioned to a fluorescent protein, great tools for	
	live-cell imaging and for dynamic investigation of sub-	
	cellular signal pathways.	
<u>Cytoskeleton</u>	A fluorescent marker (GFP, RFP or CFP) fusion with a	
<u>Imaging</u>	cellular structure protein, provides a convenient tool for	
	visualization of cytoskeletal structure	
Unstable GFP	Lentivirus express the the destabilized GFP (uGFP) which	
	provides fast turnover responses in signal pathway	
	assay and in knockdown / knockout detection	
near-infrared RFP	Ine near-infrared Red fluorescent (niRFP) expression	
	Lentiviurs provides the whole-body images with better	
	contrast and brighter images	
Fluorescent-OKF	fusion target	
	Premade lentivirus for expressing nuclear permeant	
CRE recombinase		
CRE Eln	Lentivirus expressing "LovP-GEP-Ston-LovP-PEP" or	
ColorSwtich	"ERT-GEP-Ston-ERT-REP" cassette used to monitor the	
	CRE or Flp recombination event in vivo	
	lentivirus expressing SEAP under different promoters	
SEAP Reporter	(TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal	
<u></u>	pathway responsive promoter),	
	Premade lentivirus expressin TetR (tetracycline	
TetR Repressor	regulator) protein, the repressor protein for the	
	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,	
<u>iPS factors</u>	NANOG, OCT4, SOX2, FLF4) factors with different	
· -	fluorescent and antibitoic markers	
LacZ expression	Express different full length β- galactosidase	
	(lac2) with different selection markers	
No antico a sector l	Premade negative control lentivirus with different	
<u>Inegative control</u>	markers: serves as the negative control of lentivurs	
ientiviruses	treatment, for validation of the specificity of any	



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Product Category	Product Description (please click into each category's page)	
	lentivirus target expression effects.	
Other Enzyme	Ready-to-use lentivirus, expressing a specific enzymes	
expression	with different selection markers.	
<u>Ultra titer</u>	Ultra-titer lentivirus used for the hard-to-transduced	
<u>lentivirus</u>	cells and for in vivo manipulation of sperm cells, or stem	
	cells.	