GenTarget Inc

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Pre-made unstable GFP Lentivirus

Catalog#	Product Name	Amounts
LVP1108	**uGFP** (TetCMV-**Puro**) Lentivirus	$1x10^7$ IFU/ml x 200ul (in DEME medium with 10x polybrene)
LVP1109	**uGFP** (TetCMV-**Bsd**) Lentivirus	
LVP1110	**uGFP** (TetCMV-**Neo**) Lentivirus	
LVP1108-PBS	**uGFP** (TetCMV-**Puro**) Lentivirus	$1x10^8$ IFU/ml x 200ul (concentrated virus provided in PBS)
LVP1109-PBS	**uGFP** (TetCMV-**Bsd**) Lentivirus	
LVP1110-PBS	**uGFP** (TetCMV-**Neo**) Lentivirus	

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 **TetR Lentivirus**. The TetCMV driven expression of uGFP is first repressed by TetR, then induced by adding of tetracycline.

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.

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 self-hydrolysis tag, destabilization tag or degradation domain. Gentarget's uGFP (click to see

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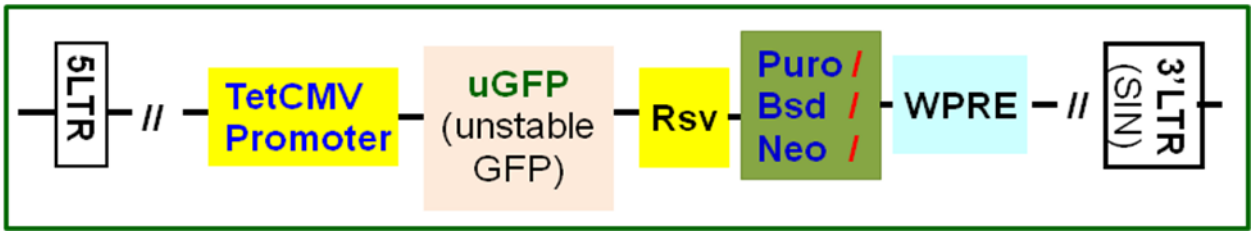
sequence) was made by fusing with a segment of mouse ornithine decarboxylase as the destabilize domain. It shows an *in vivo* half-life of about **2 hours**.

uGFP lentivirus were driven by an optional inducible 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# **HT-pack**) into 293T cells (cat# **TLV-C**). The pre-made lentiviral particles are VSV-G pseudotyped viruses. Each lot of virus is validated and quality is guaranteed.

Particles are provided in two formats:

- Regular particles in DMEM medium with 10% FBS and 60 μg/ml polybrene (10 x stock)
- Particles concentrated and buffer exchanged into PBS for *in vivo* use

For general questions about our ready-to-use lentiviral particles, please see FAQ for pre-made lentiviral particles (.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 our website for details.

Transduction Protocols (How to use the product):

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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 x 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, CO_2 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 ^oC 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 CO^2 incubator if required.

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Measure cell density (not grow over 3 million/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. 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.

Transduction Example:

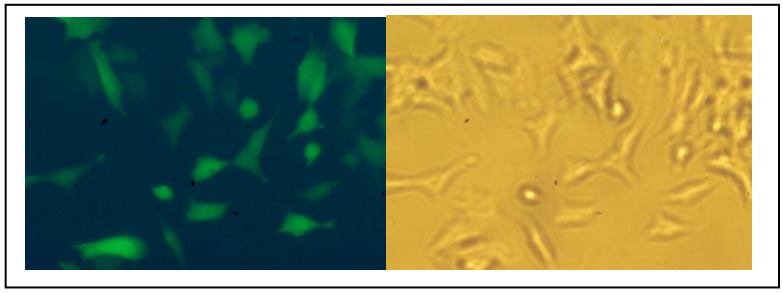

Figure 1: uGFP Expression in Hela cells. Hela cells were transduced with 50ul of Pre-made uGFP lentivirus (#LVP1108) in 24-well plate. GFP signal was visualized at 72 hours after transduction (**GFP filter: ~Ex450-490 ~Em525).**

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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:

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

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 product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Lentivirus for all kinds of pathway 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-T, TCR-T, Assay cell lines, and Cell Antigens & Receptors.
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research
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	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.

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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.
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, 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
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 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 (niRFP) expression Lentiviurs 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 flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.

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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.