



## Pre-made unstable GFP Lentivirus

Catalog#	Product Name	Amounts
<a href="#">LVP1108</a>	uGFP (TetCMV- <b>Puro</b> ) Lentivirus	1x10 <sup>7</sup> IFU/ml x 200ul ( in DEME medium with 10x polybrene)
<a href="#">LVP1109</a>	uGFP (TetCMV- <b>Bsd</b> ) Lentivirus	
<a href="#">LVP1110</a>	uGFP (TetCMV- <b>Neo</b> ) Lentivirus	
<a href="#">LVP1108-PBS</a>	uGFP (TetCMV- <b>Puro</b> ) Lentivirus	1x10 <sup>8</sup> IFU/ml x 200ul ( concentrated virus provided in PBS)
<a href="#">LVP1109-PBS</a>	uGFP (TetCMV- <b>Bsd</b> ) Lentivirus	
<a href="#">LVP1110-PBS</a>	uGFP (TetCMV- <b>Neo</b> ) 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.

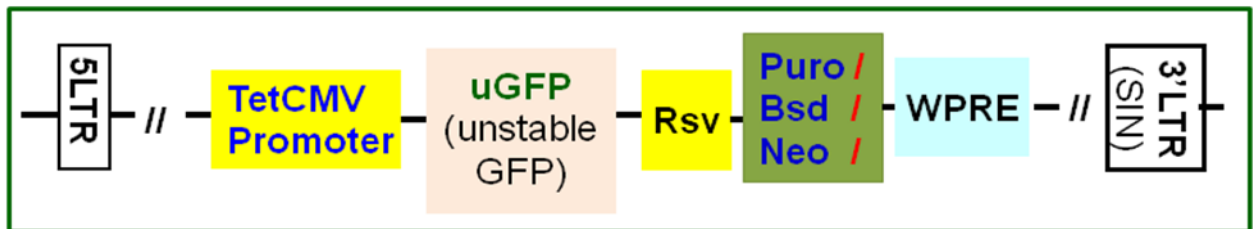
The green fluorescent protein (GFP) is a widely used reporter, provide an easy detection in living cells. However, it is a very stable protein and accumulated in cells with long half-live, which limits its application that requires fast turnover responses in signal pathway assay and in knockdown / knockout 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



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 selection marker (Puromycin, Blastidicin 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 \(How to use the product\):](#)**

#### **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  $\mu$ 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  $0.5 \times 10^5$ /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, CO<sub>2</sub> 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 cytometer (FACS or Guava).

### Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance.

## **2) Transduction Protocol for Suspension Cells:**

Grow cells in complete suspension culture medium; use a shaking flask in a CO<sub>2</sub> 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  $1 \times 10^6$  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  $\mu$ 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 CO<sub>2</sub> incubator.

### Day 2:

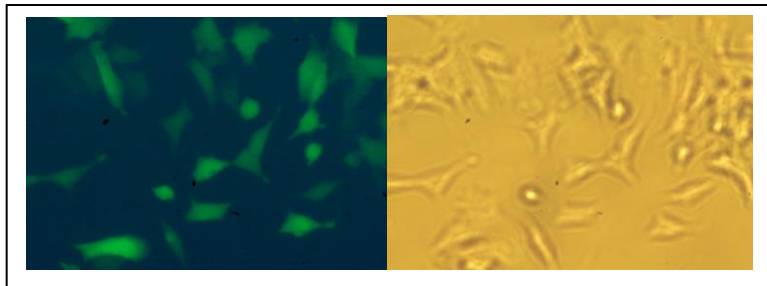
At 24 hours after transduction, add an equal amount of fresh medium containing relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO<sub>2</sub> incubator.

### 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.

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### Transduction Example:



**Figure 1: uGFP Expression in HeLa cells.** HeLa cells were transduced with 50 $\mu$ l 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.

## Related Products: GenTarget's Pre-made lentivirus Products:

<b>Lentivirus Category</b> (click to see)	<b>Product Description</b>
<a href="#">Target Expression</a>	Premade lentivirus express a <b>human, mouse or rat</b> gene with Fluorescent-Antibiotic fusion dual selection.
<a href="#">Luciferase expression</a>	Premade lentivirus express all kinds of luciferase: <b>firefly; Renilla; Cypridina; Red-Luc; Nano-Luc</b> , with different fluorescent and antibiotic selection.
<a href="#">Fluorescent markers</a>	Preamde lentivirus express human codon optimized fluorescent protein, <b>GFP / RFP / CFP / BFP / YFP/niRFP /unstable GFP, etc.</b>
<a href="#">Cytoskeleton Imaging</a>	Fluorescent ( <b>GFP / RFP/ CFP</b> ) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin)
<a href="#">Cell Organelle imaging</a>	Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging.
<a href="#">CRISPR /hu CAS9</a>	Preamde lentivirus express humanzied wild-type <b>Cas9</b> endonuclease for genomic editing by <b>CRISPR</b>
<a href="#">Fluorescent Fusion target</a>	Lentivirus express the " <b>Fluorescent-Target</b> " fusion proteins. A desired target is fused to <b>Green, Blue, Red,</b> or <b>Cyan</b> Fluorescent Protein, demonstrating the target's functionality and localization
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different flurescent and antibiotic markers.
<a href="#">LoxP ColorSwitch</a>	Premade lentivirus expressing "LoxP- <b>GFP</b> -Stop-LoxP- <b>RFP</b> " cassette, used to monitor the CRE recombination event in vivo.
<a href="#">SEAP Reporter</a>	<b>SEAP</b> (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter.
<a href="#">TetR repressor expression</a>	Premade lentivirus expressin <b>TetR</b> (tetracycline regulator) protein, the repressor protein for the inducible expression system.



<a href="#">rtTA Expression</a>	Lentivirus express the reverse tetracycline transcription activator gene, rtTA-M2 with different selection.
<a href="#">Pathway Reporter</a>	Different Report lentivirus ( <b>Luc, RFP, GFP, SEAP</b> ) under a pathway specific response promoter.
<a href="#">Cell Immortalization</a>	Comprehensive lentivirus for cell immortalization, for different cell types.
<a href="#">Cell Specific reporter</a>	Different Report lentivirus driven by cell specific promoter.
<a href="#">Infectious Antigens</a>	Lentivirus express all kinds of infectious antigens.
<a href="#">Viral Like Particle (VLP)</a>	Lentiviral particles pseudo-typed with high density of surface envelope protein.
<a href="#">Immuno Therapy</a>	Lentivirus products for Immuno Therapy application.
<a href="#">iPS factors</a>	Premade lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FGF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.