



## Human hTERT Expression Lentivirus for Cell Immortalization

CAT#	Product Name	Amounts
<a href="#">LVP1130-Neo</a>	hTERT (CMV, Neo) lentivirus	<b>200ul,</b> (1 x 10 <sup>7</sup> IFU/mL) in DMEM medium containing 10% FBS and 10 x (60 ug/m) polybrene
<a href="#">LVP1130-Bsd</a>	hTERT (CMV, Bsd) lentivirus	
<a href="#">LVP1130-Puro</a>	hTERT (CMV, Puro) lentivirus	
<a href="#">LVP1130-Zeo</a>	hTERT (CMV, Zeo) Lentivirus	
<a href="#">LVP1130-Hygro</a>	hTERT (CMV, Hygro) Lentivirus	
<a href="#">LVP1130-GB</a>	hTERT (CMV, GFP-Bsd) lentivirus	
<a href="#">LVP1130-GP</a>	hTERT (CMV, GFP-Puro) lentivirus	
<a href="#">LVP1130-RB</a>	hTERT (CMV, RFP-Bsd) lentivirus	
<a href="#">LVP1130-RP</a>	hTERT (CMV, RFP-Puro) lentivirus	
<a href="#">LVP1131-Neo</a>	hTERT (EF1a, Neo) lentivirus	
<a href="#">LVP1131-Bsd</a>	hTERT (EF1a, Bsd) lentivirus	
<a href="#">LVP1131-Puro</a>	hTERT (EF1a, Puro) lentivirus	
<a href="#">LVP1131-Zeo</a>	hTERT (EF1a, Zeo) Lentivirus	
<a href="#">LVP1131-Hygro</a>	hTERT (EF1a, Hygro) Lentivirus	
<a href="#">LVP1131-GB</a>	hTERT (EF1a, GFP-Bsd) lentivirus	
<a href="#">LVP1131-GP</a>	hTERT (EF1a, GFP-Puro) lentivirus	
<a href="#">LVP1131-RB</a>	hTERT (EF1a, RFP-Bsd) lentivirus	
<a href="#">LVP1131-RP</a>	hTERT (EF1a, RFP-Puro) lentivirus	<b>200ul,</b> (1 x 10 <sup>8</sup> IFU/mL) in <b>PBS solution</b>
<a href="#">LVP1130-Neo-PBS</a>	hTERT (CMV, Neo) lentivirus in PBS	
<a href="#">LVP1130-Bsd-PBS</a>	hTERT (CMV, Bsd) lentivirus in PBS	
<a href="#">LVP1130-Puro-PBS</a>	hTERT (CMV, Puro) lentivirus in PBS	
<a href="#">LVP1130-Zeo-PBS</a>	hTERT (CMV, Zeo) lentivirus in PBS	
<a href="#">LVP1130-Hygro-PBS</a>	hTERT (CMV, Hygro) Lentivirus in PBS	
<a href="#">LVP1130-GB-PBS</a>	hTERT (CMV, GFP-Bsd) lentivirus in PBS	
<a href="#">LVP1130-GP-PBS</a>	hTERT (CMV, GFP-Puro) lentivirus in PBS	
<a href="#">LVP1130-RB-PBS</a>	hTERT (CMV, RFP-Bsd) lentivirus	



	<b>in PBS</b>	
<a href="#">LVP1130-RP-PBS</a>	hTERT (CMV, RFP-Puro) lentivirus in PBS	
<a href="#">LVP1131-Neo-PBS</a>	hTERT (EF1a, Neo) lentivirus in PBS	
<a href="#">LVP1131-Bsd-PBS</a>	hTERT (EF1a, Bsd) lentivirus in PBS	
<a href="#">LVP1131-Puro-PBS</a>	hTERT (EF1a, Puro) lentivirus in PBS	
<a href="#">LVP1131-Zeo-PBS</a>	hTERT (EF1a, Zeo) Lentivirus in PBS	
<a href="#">LVP1131-Hygro-PBS</a>	hTERT (EF1a, Hygro) Lentivirus in PBS	
<a href="#">LVP1131-GB-PBS</a>	hTERT (EF1a, GFP-Bsd) lentivirus in PBS	
<a href="#">LVP1131-GP-PBS</a>	hTERT (EF1a, GFP-Puro) lentivirus in PBS	
<a href="#">LVP1131-RB-PBS</a>	hTERT (EF1a, RFP-Bsd) lentivirus in PBS	
<a href="#">LVP1131-RP-PBS</a>	hTERT (EF1a, RFP-Puro) lentivirus in PBS	
<a href="#">LVP1131</a>	hTERT (EF1a) lentivirus (note: no any antibiotic selection)	200ul (1x10 <sup>7</sup> IFU/ml)
<a href="#">LVP1131-PBS</a>	hTERT (EF1a) lentivirus in PBS (note: no any antibiotic selection)	200ul (1x10 <sup>8</sup> IFU/ml)

**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*. Lentivirus can stably integrate into the transduced cells’ genome for long term expression, making it a great gene transfer agent.

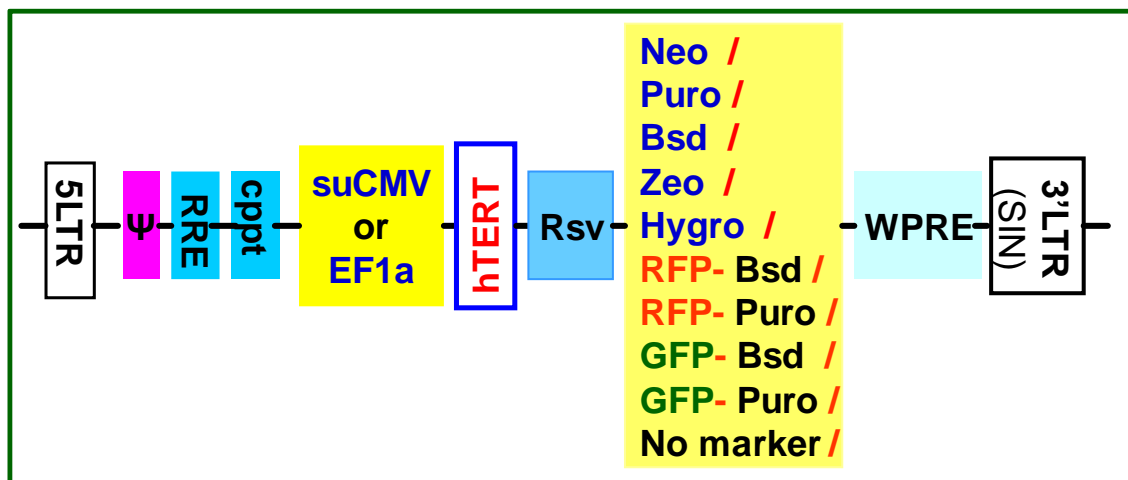
Human Telomerase Reverse Transcriptase (hTERT) plays a role in cellular senescence and also participates in chromosomal repair. When hTERT is



exogenously expressed, the cells are able to maintain telomere lengths to avoid cell senescence. Therefore, hTERT is used for primary cell immortalization for variety of cell types. For some primary cell types, the cell immortalization may requires a combination of immortalization method, like, the over-expression of both hTERT and SV40 Large T antigen. (Note: for some cell types, the over-expression hTERT may be toxic, causing cell death. If so, you have to other method, like use SV40 T antigen for the immortalization.)

hTERT expression lentivirus products are generated from GenTarget’s re-engineered lentivector system. The hTERT longest transcript (variant 1) of hTERT codon sequence ([NM\\_198253](#)), was expressed under an enhanced **CMV** (suCMV) or enhanced **EF1a** promoter. The **suCMV promoter** demonstrate the strongest expression in most cell types and the **enhanced EF1a promoter** 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**). (see **vector map scheme** below). (**Note**: we also made a product, CAT#: **LVP1131**, that does not contain any selection marker).



VSV-G pseudo-typed lentivirus are generated in 293T cell, and provided as 200 ul aliquots in two formats:

- 1) in DMEM medium containing 10% and 10x Polybrene (60 ug/ml) at titer of 1x10<sup>7</sup> IFU/ml;



2) in PBS solution at titer of  $1 \times 10^8$  IFU/ml, 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 contains a specific antibiotic resistant marker, or **antibiotic-fluorescent** fusion dual marker, used for selecting the transduced cells or generating stable cell lines by antibiotics selection or via fluorescent cell sorting. (or No any selection, CAT#: **LVP1131**).
2. The strongest [suCMV promoter](#) for high expression.
3. The enhance **EF1a promoter** is active in all cell types and do not be silenced during long-term culture.
4. The lentivirus is ready and easy to use, simply add 50ul into one well culture in 24-well plate. No need any other reagents at application.

## 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  $\mu$ l of virus into each well of 24-well-plate where cell density is 50% to 75%. After 48 to 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy. For cell selection, pass cells into medium containing corresponding antibiotic or perform fluorescence cell sorting.

#### **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:**

- 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  $-80\text{ }^{\circ}\text{C}$  for future use; virus titer will decrease by  $\sim 10\%$  for each freeze/thaw cycle.

### Day 3:

Depends on cell types, at 48~72hr post transduction, check the transduction rate by fluorescence microscopy (if applicable) or calculate the exact transduction rate by flow cytometry (FACS or Guava). Cells are ready for antibiotic selection.

### Day 3 /+:

Select transduced cells via 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  $\text{CO}_2$  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 lentivirus into the diluted cells at a ratio of: 50 to 100  $\mu\text{l}$  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  $\text{CO}_2$  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  $\text{CO}_2$  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 or select the cell by antibiotic killing, and maintain antibiotic selection to generate a stable cell line.



**Note: Filter wavelength settings:**

**GFP** filter: ~Ex450-490; ~Em525; **RFP** filter: ~Ex545; ~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. Human Molecular Genetics, Volume 8, Issue 1, 1 January 1999, Pages 137-142.
2. Current Opinion in Genetics & Development. Volume 9, Issue 1, 1 February 1999, Pages 97-103.
3. Carcinogenesis, Volume 26, Issue 5, 1 May 2005, Pages 867-874

**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 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 humanized 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, or 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 fluorescent 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>	Premde 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</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human



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<a href="#">lentivirus</a>	and mouse miRNA.
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentiviruses 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.