GenTarget Inc
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Human hTERT Expression Lentivirus for Cell Immortalization

CAT#	Product Name	Amounts
LVP1130-Neo-PBS	hTERT (CMV, **Neo**) lentivirus **in PBS**	**200ul,** (1×10^8 IFU/mL) in PBS solution with premixed Polybrene
LVP1130-Bsd-PBS	hTERT (CMV, **Bsd**) lentivirus **in PBS**	
LVP1130-Puro-PBS	hTERT (CMV, **Puro**) lentivirus **in PBS**	
LVP1130-Zeo-PBS	hTERT (CMV, **Zeo**) lentivirus in PBS	
LVP1130-Hygro-PBS	hTERT (CMV, **Hygro**) Lentivirus in PBS	
LVP1130-GB-PBS	hTERT (CMV, **GFP-Bsd**) lentivirus **in PBS**	
LVP1130-GP-PBS	hTERT (CMV, **GFP-Puro**) lentivirus **in PBS**	
LVP1130-RB-PBS	hTERT (CMV, **RFP-Bsd**) lentivirus **in PBS**	
LVP1130-RP-PBS	hTERT (CMV, **RFP-Puro**) lentivirus **in PBS**	
LVP1130-PBS	hTERT (CMV) lentivirus **in PBS** (**note**: no any antibiotic selection)	
LVP1131-Neo-PBS	hTERT (EF1a, **Neo**) lentivirus **in PBS**	
LVP1131-Bsd-PBS	hTERT (EF1a, **Bsd**) lentivirus **in PBS**	
LVP1131-Puro-PBS	hTERT (EF1a, **Puro**) lentivirus **in PBS**	
LVP1131-Zeo-PBS	hTERT (EF1a, **Zeo**) Lentivirus in PBS	
LVP1131-Hygro-PBS	hTERT (EF1a, **Hygro**) Lentivirus in PBS	
LVP1131-GB-PBS	hTERT (EF1a, **GFP-Bsd**) lentivirus **in PBS**	
LVP1131-GP-PBS	hTERT (EF1a, **GFP-Puro**) lentivirus **in PBS**	
LVP1131-RB-PBS	hTERT (EF1a, **RFP-Bsd**) lentivirus **in PBS**	
LVP1131-RP-PBS	hTERT (EF1a, **RFP-Puro**) lentivirus **in PBS**	
LVP1131-PBS	hTERT (EF1a) lentivirus **in PBS** (**note**: no any antibiotic selection)	

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

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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*. 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,** or **no any selection marker**). (see **vector map scheme** below). Lentivirus is VSV-G pseudo-typed generated in 293T cell, and provided as 200 ul aliquots

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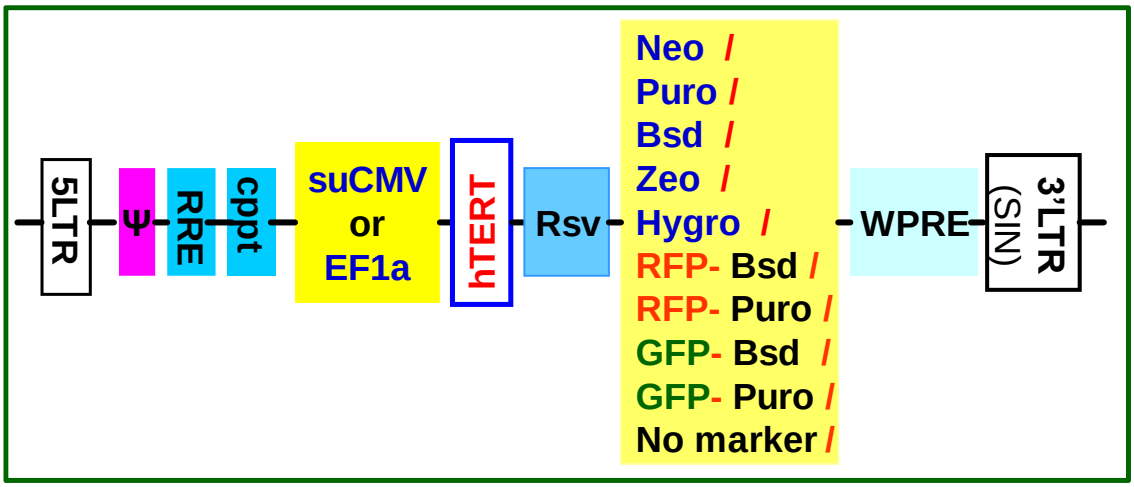

For general questions about our ready-to-use particles, please see FAQ for pre-made lentiviral particles (.pdf) on our website. (http://www.gentarget.com/pdf/FAQ-Premade-Lentiviral-particles.pdf).

2. Key features:

1) Each lentivirus 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 when desired).
2) The strongest *suCMV promoter* with the high expression for better cell immortalization success.
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 in 24-well-cell culture plate. No need for any other reagents.

3. Transduction Protocols:

Note: Pre-made lentivirus is provided as ready to use status, simply added into your cell culture. The amount of lentivirus to add depends on cell type. For most cell types, add 50 µl of virus into one well of 24-well-plate where cell density is 50% to 75%. After 72 hours (no need to change medium), check the transduction rate by fluorescence microscopy when applicable. 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 HT1080 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 °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, and select for 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).

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:

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

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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 application. In no event shall GenTarget be liable for any incidental or consequential damages in connection with 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
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
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 sub-cellular 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
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.

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Product Category	Product Description (please click into each category's page)
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwtich	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
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.