



Pre-made Reporter Lentivirus for Stem Cells

Cat#	Product Name	Amounts
LVP989-P or: LVP989-P-PBS	GATA2-GFP (Puro) Lentivirus	200ul, ~1 x 10 ⁷ IFU/mL in DMEM containing 10% FBS
LVP990-P or: LVP990-P-PBS	GATA2-RFP (Puro) Lentivirus	
LVP991-P or: LVP991-P-PBS	GATA2-Luciferase (Puro) Lentivirus	
LVP992-P or: LVP992-P-PBS	GATA2-Rluc (Puro) Lentivirus	
LVP989-B or: LVP989-B-PBS	GATA2-GFP (Bsd) Lentivirus	
LVP990-B or: LVP990-B-PBS	GATA2-RFP (Bsd) Lentivirus	
LVP991-B or: LVP991-B-PBS	GATA2-Luciferase (Bsd) Lentivirus	
LVP992-B or: LVP992-B-PBS	GATA2-Rluc (Bsd) Lentivirus	
LVP989-N or: LVP989-N-PBS	GATA2-GFP (Neo) Lentivirus	
LVP990-N or: LVP990-N-PBS	GATA2-RFP (Neo) Lentivirus	
LVP991-N or: LVP991-N-PBS	GATA2-Luciferase (Neo) Lentivirus	
LVP992-N or: LVP992-N-PBS	GATA2-Rluc (Neo) Lentivirus	
LVP989-R or: LVP989-R-PBS	GATA2-GFP (RFP) Lentivirus	
LVP991-R or: LVP991-R-PBS	GATA2-Luciferase (RFP) Lentivirus	
LVP992-R or: LVP992-R-PBS	GATA2-Rluc (RFP) Lentivirus	
LVP990-G or: LVP990-G-PBS	GATA2-RFP (GFP) Lentivirus	200ul, ~1 x 10 ⁸ IFU/mL in PBS solution
LVP991-G or: LVP991-G-PBS	GATA2-Luciferase (GFP) Lentivirus	
LVP992-G or: LVP992-G-PBS	GATA2-Rluc (GFP) Lentivirus	

Storage: <-70 °C, avoid repeat freeze/thaw cycles. Stable for >6 months.

Introduction:

Lentiviral system is a gene delivery tool using 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.

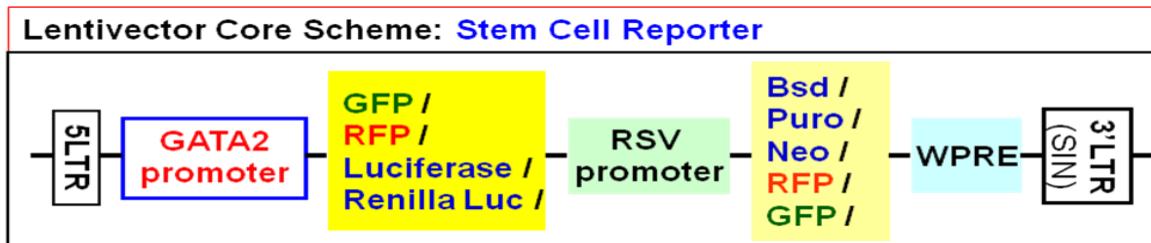
GATA2 Promoter:

The GATA2 promoter drives a transcription factor, GATA2 gene which expresses at high levels in hematopoietic stem cell, and also in nonhematopoietic embryonic stem cells. GATA2 promoter also is active in the central nervous system but may not active or has very weak promoter strength in other cell types.

Product Principle:

GenTarget developed a set of reporting lentivirus for specifically or preferably labeling Stem Cells. Those reporting lentivirus has a **luminescent report** or a **fluorescent report** under the **native GTAT2-promoter** that highly expressed in hematopoietic progenitors, including early erythroid cells, mast cells, and megakaryocytes, and also in nonhematopoietic embryonic stem cells. Those reporter lentivirus are best suitable for infecting the hematopoietic and embryonic Stem Cells, as well as for the signal pathway research on GATA2 promoter regulation.

Those reporting lentivirus also constitutively express a fluorescent selection marker or an antibiotic selection marker under the RSV promoter (Rous Sarcoma Virus Promoter) which is a moderate to strong promoter in most cell types. This selection marker is used to select the lentivirus infected cells (to generate the stable cell lines) via antibiotic killing or fluorescent cell sorting. It also provides internal reference for virus transduction efficiency when a fluorescent marker is under the RSV promoter (wherever the RSV promoter is active in assay cell type). See the scheme below for lentivector's core expression cassette.





The premade, ready-to-use reporter lentivirus provides a much easier tool to labeling or reporting for hematopoietic stem cell as well as for some nonhematopoietic embryonic stem cells *in vitro* and *in vivo* via the luciferase signal or fluorescent signal.

Lentivirus are HIV-based, pseudotyped with VSVG envelope protein, produced in 293T cells with the 3rd generation lentivirus Bio-safety features. All particles were tested to be free bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

Key Application for cell specific reporter Lentivirus:

1. Label specific cell type or create specific reporter cell line which provide a tool to monitor the specific cell type *in vitro* and *in vivo*;
2. measure the specific promoter strength in different cell types;
3. Signal pathway research on promoter specific regulation.

Product Formats:

The pre-made lentivirus provided in two formats:

1. Packaged in 10% of FBS in DMEM containing 10% FBS and 60ug/ml of polybrene (10x);
2. Particles were concentrated and buffer exchanged in PBS without any human or animal origin components. The virus in PBS are used for any cell types that requires non-serum in the culture medium, or best for the hard-to-infect cell types.

The lentivirus are ready and easy to use, simply add 50ul into one well of your cell culture in 24-well plate, and select or sort the positive transduced cells at 2-3 days post virus transduction (for sensor cell line assay). Or simply go for Estrogen receptor signal induction without the selection (for transient assay). The readout can be easily monitored by luciferase assay or via the Fluorescent microscope or readers depending on product report type.

For more details about premade particles, please see [FAQ for pre-made lentiviral particles \(.pdf\)](#).

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 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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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 $\sim 10\%$ 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.

Measure cell density (not grow over 3 million/ml), measured viability should be $> 90\%$. Dilute cells into 1×10^6 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.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525; RFP filter: ~Ex545 ~Em620;

Safety Precaution:

Gentarget lentiviral particles adapts most 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 at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

- J Neurosci Methods. 2010 May 30; 189(1): 56–64
- Blood 2012 120:1233.

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)
<u>Pathway Reporter</u>	Reporter Lentivirus for all kinds of pathway screening assays
<u>Cell Immortalization</u>	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.
<u>ImmunoOncology 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 Lentivirus</u>	CARs Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGF β ; TCRs : MART-1/ NY-ESO1/ CD1d-a-GalCer/ TRaV3-F2A-TR β V5-6;
<u>CRISPR Gene Editing</u>	Preamde lentivirus express humanized wild-type Cas9 endonuclease, the dCas9 , gRNAs, CRISPR gene editing research
<u>Epigenomic: CRISPRi and CRISPRa</u>	" dCas9-Protein " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
<u>Cell-Specific 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
<u>Infectious Antigens</u>	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
<u>Virus Like Particles (VLP)</u>	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
<u>Non-integrating LV</u>	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.



Product Category	Product Description (please click into each category's page)
shRNA Knockdown	Knockdown verified 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 expressing a human, mouse or rat gene with RFP-Blastidin 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-Latex 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 fused 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 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 Lentivirus 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 fluorescent and antibiotic markers.



Product Category	Product Description (please click into each category's page)
CRE, Flp ColorSwitch	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.