



Reporting Lentivirus for detection of TGF- β Signaling Pathway Activity

Cat#	Product Name	Amounts
LVP1714	Luc (Puro) TGF- β Pathway Lentivirus	200ul x (1 x 10 ⁸ IFU/mL)
LVP1715	GFP (Puro) TGF- β Pathway Lentivirus	
LVP1716	Luc (GFP) TGF- β Pathway Lentivirus	

Storage: Kept at -80 °C freezer, avoid repeat freeze/thaw cycles. Stable for 6 months after the receiving.

1. Product Description:

The lentiviral system is a gene delivery tool that uses lentivectors for gene expression or knockdown. GenTarget's lentivector system is based on Human Immunodeficiency Virus-1 (HIV) plasmids for these purposes. Lentivectors are used to generate lentiviral particles (lentivirus), which can be transduced into nearly all types of mammalian cells, including stem cells, primary cells, and non-dividing cells, both in vivo and in vitro. Lentiviral particles stably integrate into the genome of transduced cells, ensuring long-term expression, making them an excellent gene transfer agent.

The Transforming Growth Factor-beta (TGF- β) signaling pathway is a crucial cellular communication system. Once activated, the TGF- β proteins will bind to its receptors, which phosphorylate SMAD proteins. The phosphorylated SMAD complex translocates to the nucleus where they interact with other transcription factors to regulate the expression of target genes.

The TGF- β signaling pathway is a complex and versatile regulatory system that influences numerous cellular and physiological processes. It is involved in both normal physiological functions and pathological conditions, such as cancer and fibrosis. Its precise modulation is critical for maintaining cellular homeostasis and preventing pathological conditions [\[1\]](#).

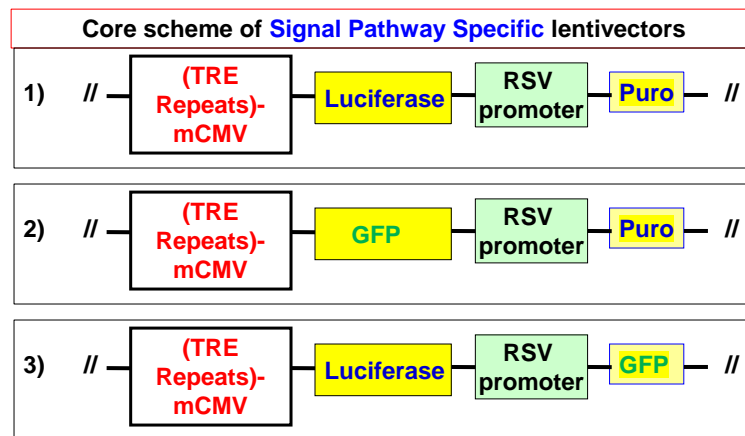
2. TGF- β pathway Lentivectors:

GenTarget developed a set of reporting lentivirus products for monitoring or detecting the TGF- β pathway's activity in any of your desired cell types. Those reporting lentivirus has a **luminescent report (Firefly-**

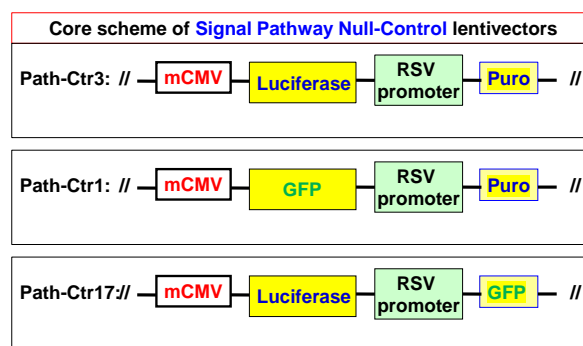


Luciferase) or a **fluorescent report (GFP)**, under the TGF- β responsive promoter of minimal CMV promoter (mCMV) containing pathway-specific multiple SMAD binding sites (5'- **AGCCAGACA**GAGGGGACTGCCC-**AGCCAGACA**GAGGGGACTGCCC 3') [2] as the transcriptional response element (**TRE**).

Those reporting lentivirus also constitutive express the **Puromycin** antibiotic selection marker or **GFP** fluorescent selection marker under a constitutive RSV promoter, which provides the selection of the transduced cells (to generate pathway specific sensor cell lines), via puromycin killing or GFP positive cell sorting. See lentivector's core scheme below.



A set of corresponding **pathway Null-Control lentivirus** were also generated. Those control use the minimal promoter that does not contain any signal pathway's TRE sequences, and will not response to pathway's induction or treatment. The pathway control lentivirus are used to set the signal reference for the specificity of pathway treatment. See the scheme below for the pathway control lentivectors.



3. Premade TGF- β signal reporting lentivirus:



Gentarget produced the replication incompetent lentivirus using 3rd-generation lentiviral packaging system. Lentivirus are HIV-based, pseudotyped with VSVG envelope protein, produced in 293T cells. All particles were tested to be free bacterial and mycoplasma contamination. Virus titers were tested lot by lot.

The ready-to-use lentivirus expresses a report: **firefly Luciferases (Luc)**, or **GFP fluorescent report** under TGF- β pathway responsive promoter. When the pathway ligands (TGF- β 1, TGF- β 2, and TGF- β 3) bind to their receptors, the final formed transcriptional regulation complex, composed of SMAD proteins, translocates to the nucleus. There, it binds to specific SMAD site in the promoter regions of the report, leading to regulated expression of the report, the Luciferase or GFP depend upon the lentivirus used.

Each lentivirus product also contains a constitutively expressed selectable marker: Puromycin (**Puro**), or **GFP** fluorescent.

The lentivirus is 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 screening cell line generation). Or simply go for signal pathway specific induction assays without the selection (for transient assay). The readout can be easily monitored by luciferase signal or via the GFP Fluorescent signal depending on the product.

The premade reporter lentivirus provides an easier, sensitive and quantitative tool to monitor activity of the signaling pathways, or screen the components that affect the pathway specific transcriptional factors. It allows to generate your reporting cell line in your desired cell type, for the study or screening of pathway specific chemicals / drug / protein in the cell-based assay.

4. Application for Pathway Reporting Lentivirus:

- 1) Create signal pathway specific cell lines for a High-throughput, live cell-based assays for signal transduction tests;
- 2) Identify or validate the signaling pathway specific drugs (drug discovery and validation);
- 3) Analyze the pathway-specific responses to proteins, peptides, or hormones;
- 4) Analyze the pathway-specific responses to gene activation, over-expression, knockdown, knockout, or mutagenesis;



- 5) Screen for pathway-specific stimulus or for the transcriptional activators that response to specific pathway's TRE elements;
 - 6) makes it easy to measure the transcriptional and post-transcription regulation in response to signal pathway stimulus.
- For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

5. Transduction Protocols (How to use the lentivirus):

Simple Protocol: 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 μ l of virus into each well of 24-well-plate where cell density is 50% to 75% (no need to worry about MOI unless you want to control the inserted copy numbers of expression cassette). After 72 hours (no need to change medium), visualize positive transduction rate by fluorescence microscopy if applicable. For stable cell line generation, pass cells into selection medium containing Puromycin, or perform GFP 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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ in a well of a 24-well plate.

Day 1:

- 1) Thaw the lentivirus products at room temperature and add the 50ul of lentivirus into one well. (Or add the amount to obtain the desired MOI. If desirable, please set up the controls by using Path-control lentivirus.
- 2) Return cells to 37°C, CO₂ incubator.

Note: If you do not use up all 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. Try to avoid freezing and thawing.

Day 3:

- 1) At ~72hr after transduction, if applicable, check the transduction rate by GFP positive cell counts under fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS).

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525;



- 2) Enrich the positive transduced cells by sorting the GFP positive cells (if applicable), or by Puromycin killing selection. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line

Day 3 +:

- 1) Grow up the positive transduced cells. The selected stable cells will be used for signal pathway assay with desired treatment. (Note: the selected Pathway Control cells serve as the non-pathway specific signal background).
- 2) Treat the cells with signal pathway inducers or inhibitors, and analyze the pathway reporter expression (GFP Fluorescent readout, or luciferase assay depend upon the products).

6. Signal pathway assay recommendations:

- 1) Treatment: the reporter's inducible expression or inhibition is dose and time dependent upon induction or treatment. You may need to optimize the best treatment amount and the time point.
- 2) Controls:
 - Pathway Null response Controls (CAT#: [Path-Ctr3](#); [Path-Ctr1](#) to [Path-Ctr17](#)): Gentarget's Pathway control lentivirus contains the minimal promoter in the same lentivector backbones as for the pathway lentivirus. The minimal promoter, demonstrated weak promoter strength in most cell types, drives the report expression which services as the signal control for pathway non-specific response.
 - Positive response controls: If applicable, apply the characterized pathway stimulus as the pathway positive induction controls, such as treated with known inducers (or inhibitors), proteins, peptide or compounds.
- 3) Make triplicates for each condition for assay reproducibility.
- 4) Assay cell number: you may need to carry out a cell titration to determine the optimal cell number for the signal reporter assay.

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

8. References:

- 1) *TGF beta signals through a heteromeric protein kinase receptor complex". Cell. 71 (6): 1003-14, 1992*
- 2) *Recent Advances in Understanding Mechanisms of TGF Beta Signaling and Its Role in Glioma Pathogenesis, pp 179-201*

9. 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.

10. **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 /TGF β ; TCRs : MART-1/ NY-ESO1/ CD1d- α -GalCer/ TR α V3-F2A-TR β V5-6;
CRISPR Gene Editing	Preamde lentivirus express humanized wild-type Cas9 endonuclease, the dCas9 , gRNAs, CRISPR gene editing



Product Category	Product Description (please click into each category's page)
	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	Lentivirus 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.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
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-miRNA 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-Blasticidin 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, mRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Lantern 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



Product Category	Product Description (please click into each category's page)
	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.
CRE recombinase	Premade lentivirus for expressing nuclear permeant CRE recombinase with different flurescent and antibiotic markers.
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 antibiotic 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 lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects.
Other Enzyme	Ready-to-use lentivirus, expressing a specific enzymes



Product Category	Product Description (please click into each category's page)
expression	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.