



Pre-made Control Lentivirus for Pathway Report Lentivirus

Cat#	Product Name	Amounts
Path-Ctr1 or Path-Ctr1-PBS	miniPro(Null)- GFP (Puro) lentivirus	200ul, ~1 x 10 ⁷ IFU/mL in DMEM containing 10% FBS Or 200ul, ~1 x 10 ⁸ IFU/mL in PBS solution
Path-Ctr2 or Path-Ctr2-PBS	miniPro(Null)- RFP (Puro) Lentiviral particles	
Path-Ctr3 or Path-Ctr3-PBS	miniPro(Null)- Luc (Puro) lentivirus	
Path-Ctr4 or Path-Ctr4-PBS	miniPro(Null)- Rluc (Puro) lentivirus	
Path-Ctr19 or Path-Ctr19-PBS	miniPro(Null)- SEAP (Puro) lentivirus	
Path-Ctr5 or Path-Ctr5-PBS	miniPro(Null)- GFP (Bsd) lentivirus	
Path-Ctr6 or Path-Ctr6-PBS	miniPro(Null)- RFP (Bsd) lentivirus	
Path-Ctr7 or Path-Ctr7-PBS	miniPro(Null)- Luc (Bsd) lentivirus	
Path-Ctr8 or Path-Ctr8-PBS	miniPro(Null)- Rluc (Bsd) lentivirus	
Path-Ctr20 or Path-Ctr20-PBS	miniPro(Null)- SEAP (Bsd) lentivirus	
Path-Ctr9 or Path-Ctr9-PBS	miniPro(Null)- GFP (Neo) lentivirus	
Path-Ctr10 or Path-Ctr10-PBS	miniPro(Null)- RFP (Neo) lentivirus	
Path-Ctr11 or Path-Ctr11-PBS	miniPro(Null)- Luc (Neo) lentivirus	
Path-Ctr12 or Path-Ctr12-PBS	miniPro(Null)- Rluc (Neo) lentivirus	
Path-Ctr21 or Path-Ctr21-PBS	miniPro(Null)- SEAP (Neo) lentivirus	
Path-Ctr13 or Path-Ctr13-PBS	miniPro(Null)- GFP (RFP) lentivirus	
Path-Ctr14 or Path-Ctr14-PBS	miniPro(Null)- Luc (RFP) lentivirus	
Path-Ctr15 or Path-Ctr15-PBS	miniPro(Null)- Rluc (RFP) lentivirus	



Path-Ctr22 or Path-Ctr22-PBS	miniPro(Null)- SEAP (RFP) lentivirus	
Path-Ctr16 or Path-Ctr16-PBS	miniPro(Null)- RFP (GFP) lentivirus	
Path-Ctr17 or Path-Ctr17-PBS	miniPro(Null)- Luc (GFP) lentivirus	
Path-Ctr18 or Path-Ctr18-PBS	miniPro(Null)- Rluc (GFP) lentivirus	

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

Product Description:

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.

The **Pathway Report Lentivirus** are a group of premade lentivirs to measure the responses to stimulus in any cell types, of a group of pathway specific response elements. In those report lentivirus, a tandem repeats of pathway specific transcription response element (TRE) is inserted into a minimal TATA promoter that drive a report expression.

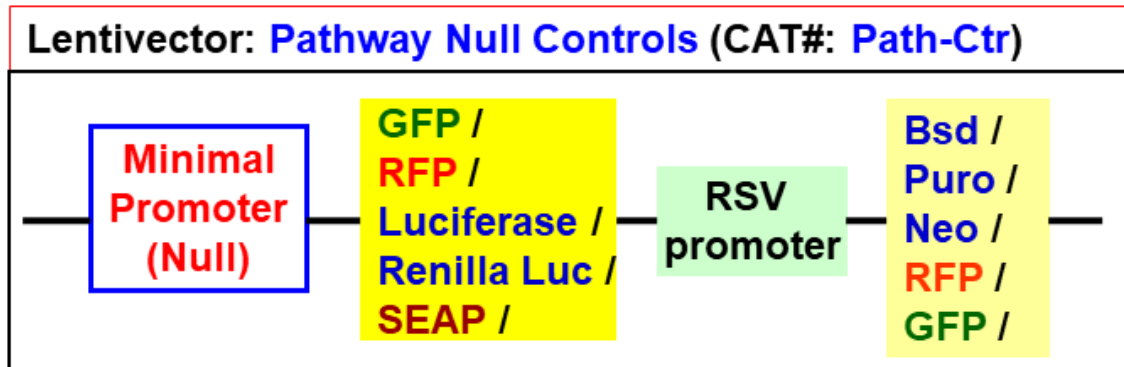
To serve as the non-response control lentivirus, a set of Pathway Null-control Lentivirus are provided by Gentarget. The pathway control lentivirus has a Null sequence inserted into the same minimal promoter. The Null-sequence does not response to any signal pathway's stimulus. Those Pathway Null-control Lentivirus are produced in the same lentivector backbone containing the same antibiotic or fluorescent marker at that in the report lentivirus. They are used to establish the Non-response control profiles to any stimulus while use Pathway Report Lentivirus.

The minimal TATA promoter after inserted with a Null-sequence, has very weak promoter strength in most cell types.

You select the Pathway control lentivirus that has the same antibiotic or fluorescent marker as the Signal Pathway Report Lentivirus does. The



antibiotic or the fluorescent marker is constitutively expressed under RSV promoter and do not affect by any signal pathway or its stimulus. See the control lentivirus's core structure scheme below.



The premade, ready-to-use reporter lentivirus provides an easier, sensitive and quantitative tool to monitor the activity of a specific signaling pathway in virtually any mammalian cell type. It also allows to generate your own reporting cell line in your desired cell type for study or screen of pathway specific gene-knockdown, over-expression, or chemical / drug/protein treatment in the cell based assay.

Pathway report control lentivirus:

The ready-to-use lentivirus expresses a report: **firefly Luciferases (Luc)**, **Renilla luciferase (RLuc)**, **GFP or RFP**, under the minimal promoter inserted with a Null-sequence. The report is expressed in minimal in most cell types and does not affected by any signal specific stimulus. Each lentivirus product also constitutively expresses a selectable marker: Blasticidin (**Bsd**), Puromycin (**Puro**), Neomycin (**Neo**), **GFP** or **RFP** fluorescent, under a separated RSV promoter.

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 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 signal induction without the selection (for transient assay).



You use this control lentivirus to establish the non-response signal profile to the desired stimulus. The report's readout can be easily monitored by luciferase assay or via the Fluorescent microscope or readers depending on product report type.

Ready-to-use luciferase lentiviral particles are 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 is good for any cell types that requires non-serum in the medium, or good for hard-to-infect cell types.

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

Transduction Protocols:

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 μ l of virus into each well of 24-well-plate where cell density is 50% to 75% (It equivalents to a MOI=50 for most cell types at such conditions). 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 for selection, 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 0.5×10^5 /ml x 0.5ml in a well of a 24-well plate.

Day 1:

- Thaw the lentivirus products at room temperature and add the appropriate amount of virus stock to obtain the desired MOI. If desired, set up the controls by using Path-control lentivirus.
- Return cells to 37°C, CO₂ incubator.



Note: Try to avoid freezing and thawing. 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.

Day 3:

At ~72hr after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava). You can now treat the cell for signal pathway assay. (Note: the Pathway Control Lentivirus serves as the non-pathway specific signal background).

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). The selected stable cells will be used for signal pathway assay with desired treatment.

Next: Treat the cell with signal pathway inducer, and analyze the pathway reporter expression (Fluorescent readout or luciferase assay).

Signal pathway assay recommendations:

- 1. Treatment:** the reporter's inducible expression 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-Ctr1](#) to [Path-Ctr18](#)): Gentarget's Pathway control lentivirus contains the minimal promoter in the same lentivector backbones. 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. Be sure to select/use the pathway control virus in the same vector backbone, i.e. having the same antibiotic marker or a fluorescent marker.
 - Positive response controls: If applicable, apply the characterized pathway stimulus as the pathway positive induction controls, such as treated with known inducer, 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.

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.

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.

Note: Filter wavelength settings:

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

Attachment: GenTarget's pre-made lentivirus product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Reporter 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 /



Product Category	Product Description (please click into each category's page)
	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 humanized 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	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-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



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



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