GenTarget Inc

7930 Arjons Drive, Suite B
San Diego, CA 92126
Phone: (858) 6788683
Fax: (800) 3804198
Email: orders@gentarget.com

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-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-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-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-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

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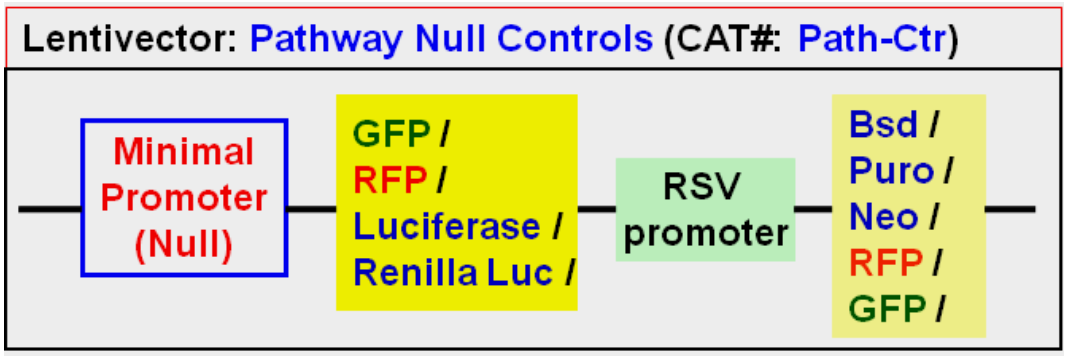
(lentivirus) that can be transduced into almost all kinds of mammalian cells, including stem cells, primary cells, and non-dividing cells both *in vivo* and *in vitro*. Lentiviral Particles stably integrate into the transduced cells' genome for long term expression, making it a great gene transfer agent.

The **Pathway Report Lentivirus** are a group of premade lentivurs 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 consitutively expressed under RSV promoter and do not affect by any signal pathway or its stimulus. See the control lentivirus's core structure scheme below.



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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).

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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 µl 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 x 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, CO_2 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.

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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. Ware 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;

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Related Products: GenTarget's pre-made lentivirus product category.

Product Category	Product Description (please click category name to see product's pages)
Pathway Report Lentivirus	Premade lentivirus expressin a luminescence or fluorescent report (firefly **Luciferase**, **Renilla** luciferase, **RFP** or **GFP** fluorescent marker) under a pathway speicfic promoter.
Human, mouse or rat ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing with **CRISPR**
TetR inducible expression repressor	Premade lentivirus expressin **TetR** (tetracycline regulator) protein, the repressor protein for the inducible expression system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
T-antigen Expression	Express **SV40 large T antigen** with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker **GFP/RFP/CFP was sub-cellular localized** in different cell organelle for living cell imaging.
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
Pre-made shRNA	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).

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lentivirus	
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
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 **specific enzymes** with different selection markers.