



## Pre-made Reporter Lentivirus for cAMP-PKA Signal Pathway

Cat#	Product Name	Amounts
<a href="#">LVP941-P</a> or: LVP941-P-PBS	CRE_TRE - <b>GFP</b> (Puro) Lentivirus	200ul, ~1 x 10 <sup>7</sup> IFU/mL in DMEM containing 10% FBS  Or 200ul, ~1 x 10 <sup>8</sup> IFU/mL in PBS solution
<a href="#">LVP942-P</a> or: LVP942-P-PBS	CRE_TRE - <b>RFP</b> (Puro) Lentivirus	
<a href="#">LVP943-P</a> or: LVP943-P-PBS	CRE_TRE - <b>Luc</b> (Puro) Lentivirus	
<a href="#">LVP944-P</a> or: LVP944-P-PBS	CRE_TRE - <b>Rluc</b> (Puro) Lentivirus	
<a href="#">LVP941-B</a> or: LVP941-B-PBS	CRE_TRE- <b>GFP</b> (Bsd) Lentivirus	
<a href="#">LVP942-B</a> or: LVP942-B-PBS	CRE_TRE- <b>RFP</b> (Bsd) Lentivirus	
<a href="#">LVP943-B</a> or: LVP943-B-PBS	CRE_TRE- <b>Luc</b> (Bsd) Lentivirus	
<a href="#">LVP944-B</a> or: LVP944-B-PBS	CRE_TRE- <b>Rluc</b> (Bsd) Lentivirus	
<a href="#">LVP941-N</a> or: LVP941-N-PBS	CRE_TRE- <b>GFP</b> (Neo) Lentivirus	
<a href="#">LVP942-N</a> or: LVP942-N-PBS	CRE_TRE- <b>RFP</b> (Neo) Lentivirus	
<a href="#">LVP943-N</a> or: LVP943-N-PBS	CRE_TRE- <b>Luc</b> (Neo) Lentivirus	
<a href="#">LVP944-N</a> or: LVP944-N-PBS	CRE_TRE- <b>Rluc</b> (Neo) Lentivirus	
<a href="#">LVP941-R</a> or: LVP941-R-PBS	CRE_TRE- <b>GFP</b> (RFP) Lentivirus	
<a href="#">LVP943-R</a> or: LVP943-R-PBS	CRE_TRE- <b>Luc</b> (RFP) Lentivirus	
<a href="#">LVP944-R</a> or: LVP944-R-PBS	CRE_TRE- <b>Rluc</b> (RFP) Lentivirus	
<a href="#">LVP942-G</a> or: LVP942-G-PBS	CRE_TRE- <b>RFP</b> (GFP) Lentivirus	
<a href="#">LVP943-G</a> or: LVP943-G-PBS	CRE_TRE- <b>Luc</b> (GFP) Lentivirus	
<a href="#">LVP944-G</a> or: LVP944-G-PBS	CRE_TRE- <b>Rluc</b> (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.

### **cAMP-PKA signaling pathways:**

Many extracellular and intracellular signaling pathways involve the **cAMP response element** (CRE) which has a general motif of 5'-TGACGTCA-3'. Many pathway signals, like cAMP, GPCR, and others, activate the CREB (cAMP Response Element Binding proteins) which in turn binds to the cAMP response motif, resulted in the transcription of the downstream gene / reporter.

The PKA enzyme is also known as cAMP-dependent enzyme because it gets activated only if cAMP is present. In cAMP/PKA pathway, PKA activate CREB which then binds to CRE motif, and induces the downstream gene expression. This pathway is critical to numerous life processes and living organisms. The CRE motif (as Transcription response element, TRE) provides a system for study of cAMP/PKA pathway's activation or inhibition.

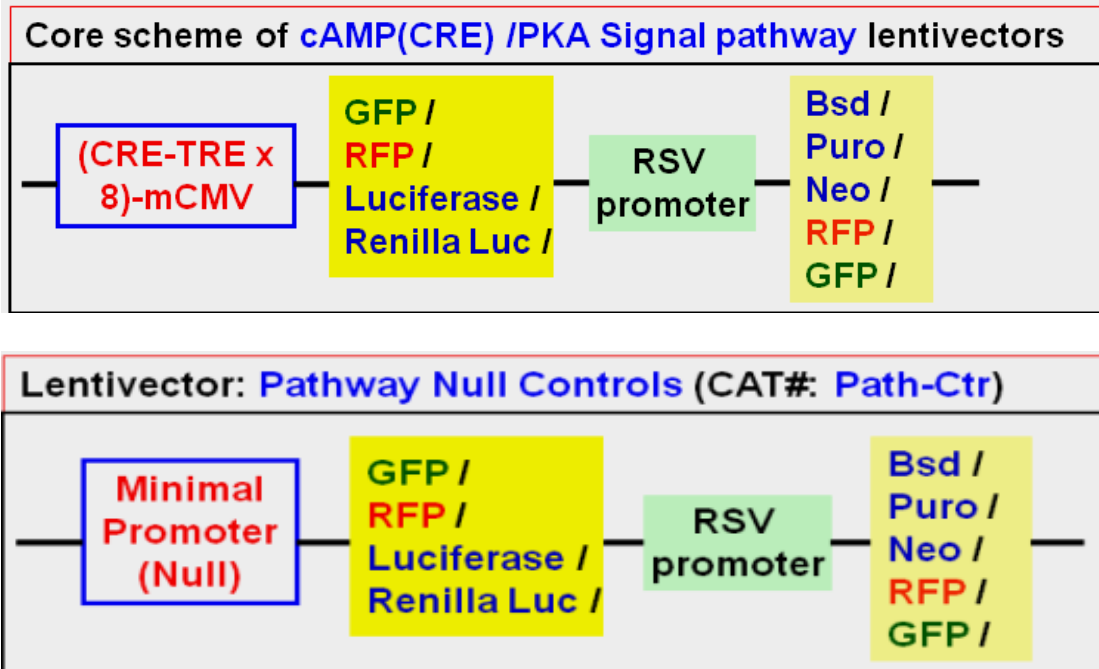
### **Product Principle:**

GenTarget developed a set of reporting lentivirus for monitoring or manipulating the CRE pathway's activity in any of your desired cell types. Those reporting lentivirus has a **luminescent report** or a **fluorescent report** under a minimal promoter that embedded with optimized tandem repeats of the CRE motif (5'-TGACGTCA-3'). When cellular cAMP level is elevated, the activated CREB will binds to the minimal promoter's TRE motif, which increases the expression of the downstream luminescent or fluorescent report. The reporter's signal can be easily and rapidly readout via plate assays (Luciferase assay or by fluorescent microscope).

Those reporting lentivirus also contains a constitutively expressed fluorescent selection marker or an antibiotic selection marker under the RSV promoter, which makes it easier to select the stably infected signal reporting cells (to generate pathway specific sensor cell lines), or provides internal reference for virus transduction efficiency when a fluorescent marker is under the RSV promoter. A set of control lentivirus use the same lentivector backbones except the minimal promoter does not contain any signal pathway's TRE sequences. The control lentivirus are used to set the reference for specificity of pathway



signal response upon treatment. See the scheme below for lentivector's core expression cassette.



The premade, ready-to-use reporter lentivirus provides a much easier tool to monitor the activity of cAMP-PKA signaling pathways 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 (for example: Forskolin), drug, protein treatment in the cell based assay.

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.

**Key Application for Pathway Signaling Lentivirus:**

1. Create signal pathway specific cell lines which can provide 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.

## 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 cAMP-PKA 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:

**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  $\mu$ l 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 (when applicable). 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  $0.5 \times 10^5$ /ml x 0.5ml in a well of a 24-well plate.



## Day 1:

- Remove the culture medium and add 0.5ml fresh, warm, complete medium.
- 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<sub>2</sub> 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.

## 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:

- Virus transduction controls: Gentarget's signal report lentivirus contains a non-inducible fluorescent marker as the internal normalization control (only applicable to the lentivirus containing a fluorescent marker under Rsv promoter). The embed internal control fluorescent signal also monitors the lentivirus transduction efficiency in assay cell types. When the internal control is not available, you can mix a regular luciferase or fluorescent marker lentivirus under a constitutive promoter (see GenTarget's [luciferase lentivirus](#) and [fluorescent lentivirus](#) products).



- No-response controls: if desired, you also can use the [Pathway-control lentivirus](#) that made in the same lentivector backbone but without any TRE in its minimal promoter. (Note: the minimal CMV promoter has no or little activity in most cell types). This control virus serves for the specificity of any treatment or as for the establishment of the basal signal profile.
  - Positive 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.

### **References:**

- Nature, 1987; 328(6126):175-178
- Trends Biochem. 2006; Sci. 31 (12): 680-6
- Mol. Cell. Biochem. 1993; 127-128: 179-86.

### **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;

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



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



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<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers:</b> serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme expression</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.