



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

Cat#	Product Name	Amounts
<a href="#">LVP941-P-PBS</a>	CRE_TRE - <b>GFP</b> ( <b>Puro</b> ) Lentivirus	200ul/each (1 x 10 <sup>8</sup> IFU/mL)
<a href="#">LVP942-P-PBS</a>	CRE_TRE - <b>RFP</b> ( <b>Puro</b> ) Lentivirus	
<a href="#">LVP943-P-PBS</a>	CRE_TRE - <b>Luc</b> ( <b>Puro</b> ) Lentivirus	
<a href="#">LVP944-P-PBS</a>	CRE_TRE - <b>Rluc</b> ( <b>Puro</b> ) Lentivirus	
<a href="#">LVP1252-PBS</a>	CRE_TRE - <b>SEAP</b> ( <b>Puro</b> ) Lentivirus	
<a href="#">LVP941-B-PBS</a>	CRE_TRE- <b>GFP</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP942-B-PBS</a>	CRE_TRE- <b>RFP</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP943-B-PBS</a>	CRE_TRE- <b>Luc</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP944-B-PBS</a>	CRE_TRE- <b>Rluc</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP1253-PBS</a>	CRE_TRE - <b>SEAP</b> ( <b>Bsd</b> ) Lentivirus	
<a href="#">LVP941-N-PBS</a>	CRE_TRE- <b>GFP</b> ( <b>Neo</b> ) Lentivirus	
<a href="#">LVP942-N-PBS</a>	CRE_TRE- <b>RFP</b> ( <b>Neo</b> ) Lentivirus	
<a href="#">LVP943-N-PBS</a>	CRE_TRE- <b>Luc</b> ( <b>Neo</b> ) Lentivirus	
<a href="#">LVP944-N-PBS</a>	CRE_TRE- <b>Rluc</b> ( <b>Neo</b> ) Lentivirus	
<a href="#">LVP1254-PBS</a>	CRE_TRE - <b>SEAP</b> ( <b>Neo</b> ) Lentivirus	
<a href="#">LVP941-R-PBS</a>	CRE_TRE- <b>GFP</b> ( <b>RFP</b> ) Lentivirus	
<a href="#">LVP943-R-PBS</a>	CRE_TRE- <b>Luc</b> ( <b>RFP</b> ) Lentivirus	
<a href="#">LVP944-R-PBS</a>	CRE_TRE- <b>Rluc</b> ( <b>RFP</b> ) Lentivirus	
<a href="#">LVP1255-PBS</a>	CRE_TRE - <b>SEAP</b> ( <b>RFP</b> ) Lentivirus	
<a href="#">LVP942-G-PBS</a>	CRE_TRE- <b>RFP</b> ( <b>GFP</b> ) Lentivirus	
<a href="#">LVP943-G-PBS</a>	CRE_TRE- <b>Luc</b> ( <b>GFP</b> ) Lentivirus	
<a href="#">LVP944-G-PBS</a>	CRE_TRE- <b>Rluc</b> ( <b>GFP</b> ) Lentivirus	

**Storage:** -80 °C, avoid repeat freeze/thaw cycles. Stable for 12 months.

### 1. 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.



## 2. 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.

## 3. Premade cAMP-PKA pathway Lentivirus:

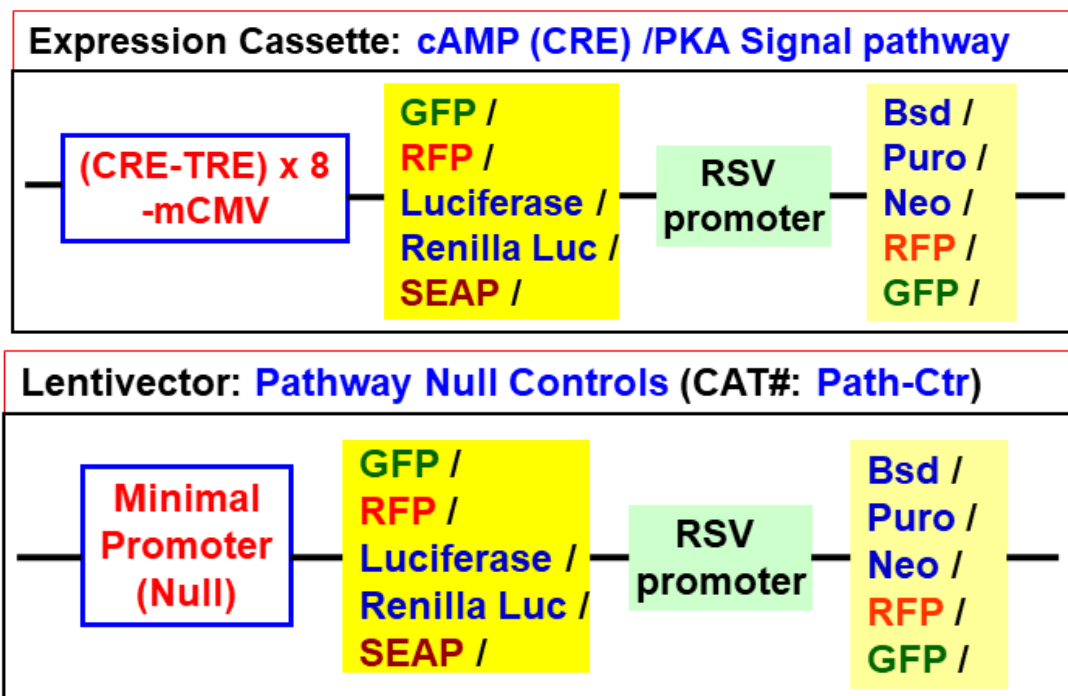
GenTarget developed a set of reporting lentivirus for monitoring or manipulating the CRE pathway's activity in any of your desired cell types. Those reporting lentivirus has a **luminescent report** (**Luciferase**, **Renilla Luc**) or a **fluorescent report** (**GFP**, **RFP**) or a secreted **SEAP** 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 luciferase signal can be measured via Luciferase assay. The fluorescent reporter can be detected via its fluorescent signal. The SEAP secreted into cell culture supernatant, which allows to determine reporter activity without disturbing the cells, does not require the preparation of cell lysates and can be used for kinetic studies.

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.

The lentivirus are 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).

#### 4. 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.

#### 5. 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  $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$  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 (or simply add 50ul into one well in 24w/p).
- 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 48~72hr 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).

## 6. 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:

- (1) 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).
- (2) 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.
- (3) 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.



- (4) Make triplicates for each condition for assay reproducibility.
- (5) 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 adapt 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) Nature, 1987; 328(6126):175-178
- 2) Trends Biochem. 2006; Sci. 31 (12): 680-6
- 3) Mol. Cell. Biochem. 1993; 127-128: 179-86.

## 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.

### Note: Filter wavelength settings:

**GFP filter:** ~Ex450-490 ~Em525;

**RFP filter:** ~Ex545 ~Em620;

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

<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Pathway Reporter</a>	Reporter Lentivirus for all kinds of pathway screening assays
<a href="#">Cell Immortalization</a>	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.



Product Category	Product Description (please click into each category's page)
<a href="#">ImmunoOncology Research</a>	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;
<a href="#">CAR-T, TCR Lentivirus</a>	<b>CARs</b> Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; <b>TCRs</b> : MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRaV3-F2A-TRβV5-6;
<a href="#">CRISPR Gene Editing</a>	Preamde lentivirus express humanized wild-type <b>Cas9</b> endonuclease, the <b>dCas9</b> , gRNAs, <b>CRISPR</b> gene editing research
<a href="#">Epigenomic: CRISPRi and CRISPRa</a>	" <b>dCas9-Protein</b> " fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
<a href="#">Cell-Specific Reporter</a>	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
<a href="#">Infectious Antigens</a>	Lentivirus that express all kinds of infectious antigens with C-term 6His-tag.
<a href="#">Virus Like Particles (VLP)</a>	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
<a href="#">Non-integrating LV</a>	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
<a href="#">shRNA Knockdown</a>	Knockdown verified and customized shRNA lentivirus for target knockdown,
<a href="#">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.
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific anti-miRNA cassette.
<a href="#">Human and mouse ORFs</a>	Premade lentivirus expressin a <b>human, mouse or rat</b> gene with RFP-Blasticidin fusion dual markers.





<b>Product Category</b>	<b>Product Description (please click into each category's page)</b>
<a href="#">Luciferase expression</a>	Premade lentivirus for all kinds of luciferase protein expression: <b>firefly and Renilla, Red-Luc and more</b> , with different antibiotic selection markers.
<a href="#">Fluorescent Markers</a>	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
<a href="#">Luminescent Imaging</a>	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
<a href="#">Sub-cellular Imaging</a>	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.
<a href="#">Cytoskeleton Imaging</a>	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
<a href="#">Unstable GFP</a>	Lentivirus express the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
<a href="#">near-infrared RFP</a>	The near-infrared Red fluorescent (niRFP) expression Lentiviruses provides the whole-body images with better contrast and brighter images
<a href="#">Fluorescent-ORF fusion</a>	Pre-made lentivirus expression a " <b>GFP/RFP/CFP-ORF</b> " fusion target.
<a href="#">CRE recombinase</a>	Premade lentivirus for expressing <b>nuclear permeant CRE</b> recombinase with different fluorescent and antibiotic markers.
<a href="#">CRE, Flp ColorSwitch</a>	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.
<a href="#">SEAP Reporter</a>	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
<a href="#">TetR Repressor</a>	Premade lentivirus expressing TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
<a href="#">rtTA Expression</a>	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF





Product Category	Product Description (please click into each category's page)
	inducible system.
<a href="#">iPS factors</a>	Premade lentivirus for human and mouse iPS ( <b>Myc, NANOG, OCT4, SOX2, FLK4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<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 a specific enzymes with different selection markers.
<a href="#">Ultra titer lentivirus</a>	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.