GenTarget Inc

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Reporter Lentivirus for MAPK/ERK Signal Pathway

Cat#	Product Name	Amounts
LVP957-P-PBS	SRE-**GFP** (Puro) Lentivirus	200ul/each (1 x 10^8 IFU/mL), containing 10x of polybrene. Or 200ul, ~1 x 10^8 IFU/mL in PBS solution
LVP958-P-PBS	SRE-**RFP** (Puro) Lentivirus	
LVP959-P-PBS	SRE-**Luc** (Puro) Lentivirus	
LVP960-P-PBS	SRE-**Rluc** (Puro) Lentivirus	
LVP1284-PBS	SRE-**SEAP** (Puro) Lentivirus	
LVP957-B-PBS	SRE- **GFP** (Bsd) Lentivirus	
LVP958-B-PBS	SRE- **RFP** (Bsd) Lentivirus	
LVP959-B-PBS	SRE- **Luc** (Bsd) Lentivirus	
LVP960-B-PBS	SRE- **Rluc** (Bsd) Lentivirus	
LVP1285-PBS	SRE-**SEAP** (Bsd) Lentivirus	
LVP957-N-PBS	SRE- **GFP** (Neo) Lentivirus	
LVP958-N-PBS	SRE- **RFP** (Neo) Lentivirus	
LVP959-N-PBS	SRE- **Luc** (Neo) Lentivirus	
LVP960-N-PBS	SRE- **Rluc** (Neo) Lentivirus	
LVP1286-PBS	SRE-**SEAP** (Neo) Lentivirus	
LVP957-R-PBS	SRE- **GFP** (RFP) Lentivirus	
LVP959-R-PBS	SRE- **Luc** (RFP) Lentivirus	
LVP960-R-PBS	SRE- **Rluc** (RFP) Lentivirus	
LVP1287-PBS	SRE-**SEAP** (RFP) Lentivirus	
LVP958-G-PBS	SRE- **RFP** (GFP) Lentivirus	
LVP959-G-PBS	SRE- **Luc** (GFP) Lentivirus	
LVP960-G-PBS	SRE- **Rluc** (GFP) 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.

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2. MAPK/ERK signaling pathways:

The mitogen-activated protein kinase (MAPK, also called ERK) pathway (also known as the Ras-Raf-MEK-ERK pathway) involves cell growth and differentiation and in the development of many cancers. Activated Ras activates the protein kinase activity of RAF kinase. RAF kinase phosphorylates and activates MEK (MEK1 and MEK2). MEK phosphorylates and activates MAPK. MAPK regulates the activities of several transcription factors. The MAPK phoshorylates Elk-1 protein which forms a complex with Serum Response Factor (SRF) and Ternary Complex Factor (TCR). This complex binds to Serum Response Element (**SRE**) sequence motif, causing transcriptional activation of target genes.

3. Premade Reporter Lentivirus for MAPK/ERK pathways:

GenTarget developed a set of reporting lentivirus to monitor MAPK/ERK signaling pathway. Those reporting lentivirus has a **luminescent report (Luciferase, Renilla Luc)** or a **fluorescent report (GFP, RFP)** or a secreted **SEAP** report, under a minimal CMV promoter (mCMV) that embedded with optimized tandem repeats of serum response element (**SRE**) sequence motif (5'- GGATGTCCATATTAGGA; CCAAAAAAGG). When transcription factors bind to SRE, the downstream reporter is expressed as the result of activation for the minimal promoter. This pathway lentivirus can be verified by FBS treatment.

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.

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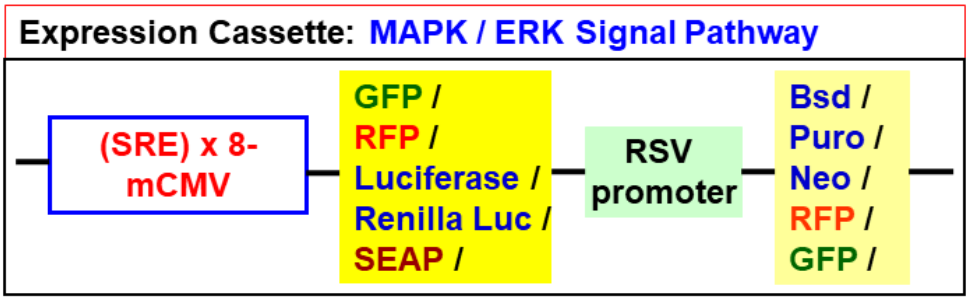

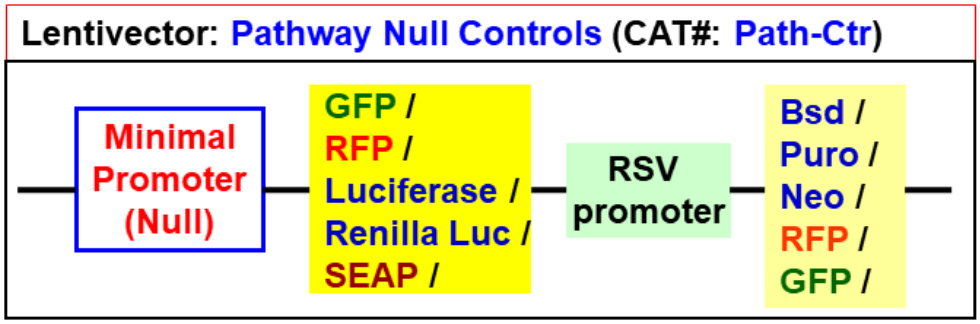

The premade, ready-to-use reporter lentivirus provides a much easier tool to monitor the activity of MAPK / ERK signaling pathways in virtually any mammalian cell type. It also allows to generate your own reporting cell line in your desired cell type for study or screen of pathway specific gene-knockdown, over-expression, or chemical / 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.

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;

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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 µl 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 x 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_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 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:

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.

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-

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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.

8. References:

1) The Biochemical Journal. 2005; 392 (Pt 2): 249–61.
2) Recent Pat Anticancer Drug Discov. 2009; 4(1):28-35.
3) Biochim. Biophys. Acta. 2007; 1773 (8): 1263–84.

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.

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 /TGFβ; **TCRs**: MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;

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CRISPR Gene Editing	Preamde lentivirus express humanzied 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	Llentivirus 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 verifeid 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 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

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Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned 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 ColorSwtich	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 antibitoic 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 lentivurs treatment, for validation of the specificity of any

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	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.