Pre-made Reporter Lentivirus for JNK / AP1 Signal Pathway

Cat#	Product Name	Amounts
LVP953-P or: LVP953-P-PBS	AP1-**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
LVP954-P or: LVP954-P-PBS	AP1-**RFP** (Puro) Lentivirus	
LVP955-P or: LVP955-P-PBS	AP1-**Luc** (Puro) Lentivirus	
LVP956-P or: LVP956-P-PBS	AP1-**Rluc** (Puro) Lentivirus	
LVP953-B or: LVP953-B-PBS	AP1- **GFP** (Bsd) Lentivirus	
LVP954-B or: LVP954-B-PBS	AP1- **RFP** (Bsd) Lentivirus	
LVP955-B or: LVP955-B-PBS	AP1- **Luc** (Bsd) Lentivirus	
LVP956-B or: LVP956-B-PBS	AP1- **Rluc** (Bsd) Lentivirus	
LVP953-N or: LVP953-N-PBS	AP1- **GFP** (Neo) Lentivirus	
LVP954-N or: LVP954-N-PBS	AP1- **RFP** (Neo) Lentivirus	
LVP955-N or: LVP955-N-PBS	AP1- **Luc** (Neo) Lentivirus	
LVP956-N or: LVP956-N-PBS	AP1- **Rluc** (Neo) Lentivirus	
LVP953-R or: LVP953-R-PBS	AP1- **GFP** (RFP) Lentivirus	
LVP955-R or: LVP955-R-PBS	AP1- **Luc** (RFP) Lentivirus	
LVP956-R or: LVP956-R-PBS	AP1- **Rluc** (RFP) Lentivirus	
LVP954-G or: LVP954-G-PBS	AP1- **RFP** (GFP) Lentivirus	
LVP955-G or: LVP955-G-PBS	AP1- **Luc** (GFP) Lentivirus	
LVP956-G or: LVP956-G-PBS	AP1- **Rluc** (GFP) Lentivirus	

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

Product Description:

Lentiviral particles or lentivirus is a gene delivery tool produced from lentivectors for gene expression or knockdown. AMSBIO's lentivector system are 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 cell's genome for long term expression, making it a great gene transfer agent.

JNK /AP1 signaling pathways:

The stress-activated protein kinase (SAPK), Jun N-terminal kinase (JNK) family (including MAP Kinases) is activated by variety of environmental stresses. This pathway is responsible for the phosphorylation and activation of Jun. During the activation, the MAPKs activate/phosphorylate JNKs which in turn activate transcription factors in nucleus. The activated or phosphorylated transcription factors (such as c-Jun JunB and JunD) form a protein complex with Fos protein family (c-Fos, Fos B, Fra-1 and Fra-2). The complex (the activator protein-1, AP1) binds to its response element sequence and induces the target gene transcription.

Product Principle:

AMSBIO has developed a set of reporter lentivirus to monitor JNK/AP1 signaling pathway. The reporter lentivirus has a **luminescent reporter** or a **fluorescent reporter** under a minimal CMV promoter (mCMV) that is embedded with optimized tandem repeats of AP1 Responsive Element (AP1-RE) sequence motif (5'- TGAGTCAG). When transcription factors bind to AP1 responsive sequence (the transcriptional response element), the downstream reporter is expressed as the result of activation of the minimal promoter. The reporter's signal can be easily and rapidly detected via plate assays (luciferase assay, fluorescent microscope or by FACS sorter).

The reporter lentivirus also constitutively expresses a fluorescent selection marker or an antibiotic selection marker under the RSV promoter, which makes it easier to select the stably transduced reporter 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 control lentivirus uses the same lentivector backbone except the minimal promoter does not contain any signal pathway's TRE sequences. The control lentivirus is used to set the reference for specificity of the pathway signal response upon treatment. See the scheme below for lentivector's core expression cassette.

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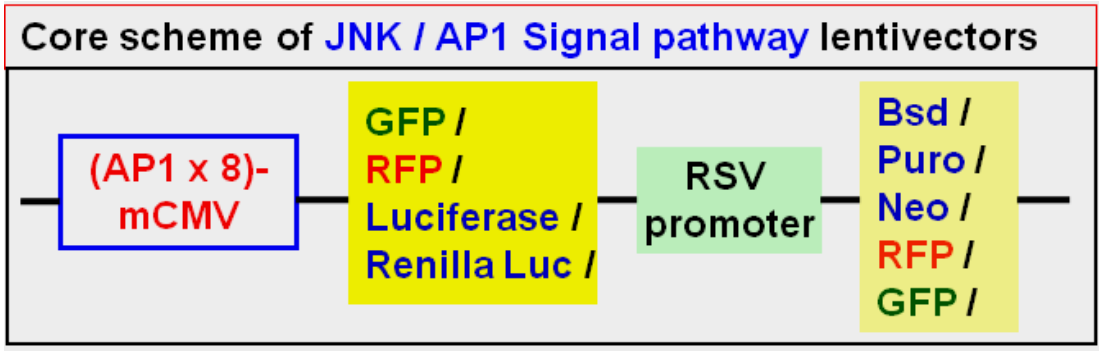

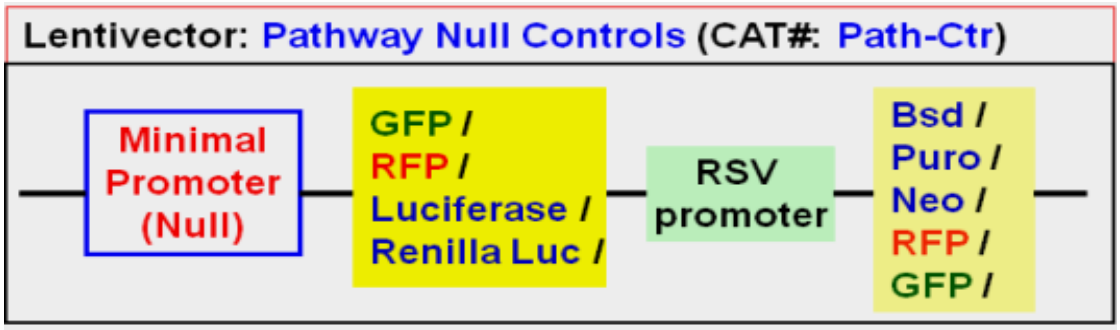

The premade, ready-to-use reporter lentivirus provides a much easier tool to monitor the activity of MAPK / JNK / AP1 signaling pathways in virtually any mammalian cell type. It also allows you to generate reporter cell line in your desired cell type for studying or screening the pathway specific gene-knockdown, over-expression, or chemical / drug /protein treatment in the cell based assay. This JNK/AP1 pathway can be tested/ simulated by Phorbol 12-Myristate 13-Acetate (PMA).

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 titer is tested on lot by lot basis.

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;

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6. Easy to measure 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 60 µg/ml of polybrene (10x);
2. Particles were concentrated and buffer exchanged in PBS without any human or animal origin components. The virus in PBS can be used for any cell type that requires no serum in the culture medium, or is best used for the hard-to-infect cell types.

For general questions about our ready-to-use particles, please see FAQ for pre-made lentiviral particles http://www.amsbio.com/FAQ-Premade-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 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. For stable cell line generation, passage cells into medium containing antibiotic or perform fluorescence cell sorting followed by antibiotic selection.

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 all of the 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 start treatment of cells 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 literature on generation of stable cell lines).

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

Controls:

Virus transduction controls: AMSBIO's signaling reporter lentivirus contains a non-inducible fluorescent marker as an internal normalization control (only applicable to the lentivirus containing a fluorescent marker under Rsv promoter). The embedded internal control fluorescent signal also monitors the lentivirus transduction efficiency in assay cell types. When the internal control is not available, you can use a regular luciferase or fluorescent marker lentivirus under a constitutive promoter.

No-response controls: if desired, you also can use the Pathway-control lentivirus that is 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 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 treatment with known inducers, proteins, peptide or compounds.

Make triplicates for each condition for assay reproducibility.

Assay cell number: you may need to carry out a cell titration to determine the optimal cell number for the signal reporter assay.

Note: Filter wavelength settings:

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

Safety Precaution:

Amsbio lentiviral particles have adopted the most 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 gloves at all times when handling lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. BioTechniques 38:891-894(June 2005);
2. THE JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 279, No. 5, Issue of January 30, pp. 3212–3217, 2004;
3. Biosci. Biotechnol. Biochem., 68(3), 565-5570, 2004;
4. Annu Rev Microbiol. 1994;48:345-69.
5. Microbiol Mol Biol Rev. 2005 Jun;69(2):326-56.

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6. APPLIED AND ENVIRONMENTAL MICROBIOLOGY, July 2005, p. 3427–3432;
7. Molecular & Biochemical Parasitology 155 (2007) 167–171;
8. Biosci. Biotechnol. Biochem., 68(3), 565-570, 2004;
9. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors link
10. CDC guidelines for Lab Biosafety levels link

Warranty:

This product is warranted to meet its quality as described when used accordance with its instructions. Amsbio disclaims any implied warranty of this product for particular application. In no event shall Amsbio be liable for any incidental or consequential damages in connection with the products. Amsbio's sole remedy for breach of this warranty should be, at Amsbio's option, to replace the products.

These products are provided for research use only!

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