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Pre-made Reporter Lentivirus for Antioxidant Signal Pathway

Cat#	Product Name	Amounts
LVP981-P or: LVP981-P-PBS	ARE-**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
LVP982-P or: LVP982-P-PBS	ARE-**RFP** (Puro) Lentivirus	
LVP983-P or: LVP983-P-PBS	ARE-**Luc** (Puro) Lentivirus	
LVP984-P or: LVP984-P-PBS	ARE-**Rluc** (Puro) Lentivirus	
LVP981-B or: LVP981-B-PBS	ARE- **GFP** (Bsd) Lentivirus	
LVP982-B or: LVP982-B-PBS	ARE- **RFP** (Bsd) Lentivirus	
LVP983-B or: LVP983-B-PBS	ARE- **Luc** (Bsd) Lentivirus	
LVP984-B or: LVP984-B-PBS	ARE- **Rluc** (Bsd) Lentivirus	
LVP981-N or: LVP981-N-PBS	ARE- **GFP** (Neo) Lentivirus	
LVP982-N or: LVP982-N-PBS	ARE- **RFP** (Neo) Lentivirus	
LVP983-N or: LVP983-N-PBS	ARE- **Luc** (Neo) Lentivirus	
LVP984-N or: LVP984-N-PBS	ARE- **Rluc** (Neo) Lentivirus	
LVP981-R or: LVP981-R-PBS	ARE- **GFP** (RFP) Lentivirus	
LVP983-R or: LVP983-R-PBS	ARE- **Luc** (RFP) Lentivirus	
LVP984-R or: LVP984-R-PBS	ARE- **Rluc** (RFP) Lentivirus	
LVP982-G or: LVP982-G-PBS	ARE- **RFP** (GFP) Lentivirus	
LVP983-G or: LVP983-G-PBS	ARE- **Luc** (GFP) Lentivirus	
LVP984-G or: LVP984-G-PBS	ARE- **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 (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.

Antioxidant signaling pathways:

A major mechanism in the cellular defense against oxidative or electrophilic stress is activation of the Nrf1/Nrf2-antioxidant response signaling pathway, which regulates the expression of genes (antioxidant proteins) involved in protecting cells from oxidative damage.

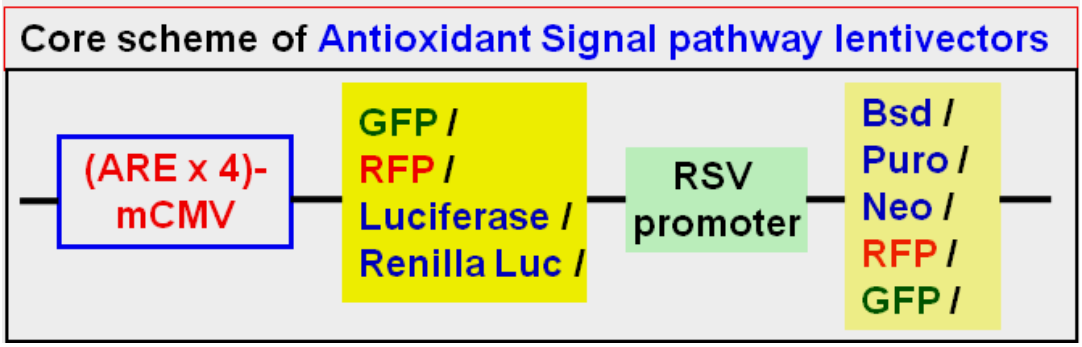
Nrf2 (NF-E2-related factor 2) is ubiquitously expressed in most tissues and is continuously degraded in the cytosol under normal oxygen conditions via its inhibitor Keap1. Keap1 contains several cysteine residues that act as redox sensors. Upon changes in the cellular oxygen environment, these cysteine residues are oxidised. As a result, Keap1 undergoes a conformational change and releases Nrf2, which is translocated into the nucleus. In the nucleus, Nrf2 forms a heterodimer with small maf proteins and binds to Nrf1/Nrf2 Response Element, antioxidant response element (**ARE**) that found in the promoter regions of many antioxidant enzymes, including thioredoxin and thioredoxin reductase, up-regulating their expression.

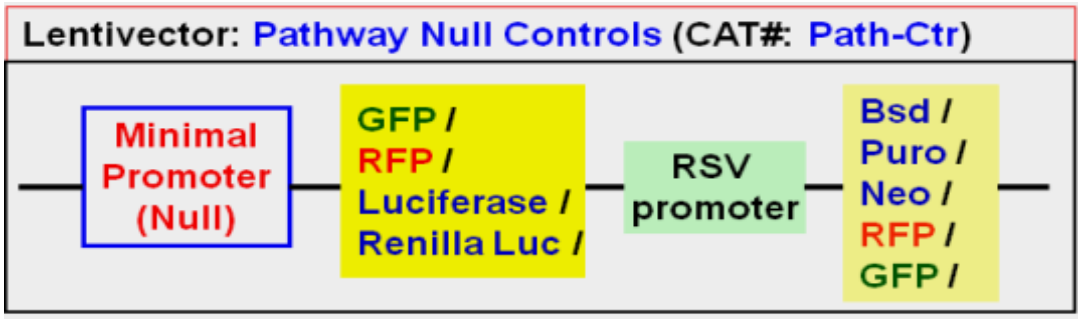
Product Principle:

GenTarget developed a set of reporting lentivirus to monitor the transcriptional activity of the Antioxidant response pathway, for both increases and decreases in the transcriptional activity of Nrf1 and Nrf2. Those reporting lentivirus has a **luminescent report** or a **fluorescent report** under a minimal CMV promoter (mCMV) that embedded with optimized tandem repeats of Nrf1/Nrf2 Response Element or " antioxidant response element" (**ARE**) sequence motif (5'-TCACAGTGACTCAGCAAAATT). When Nrf2 / Nrf1 binds to Nrf2-RE sequence, the downstream reporter is expressed as the result of activation for the minimal promoter. This pathway can be verified by DL-Sulforaphane stimulation. The reporter's signal can be easily and rapidly readout via plate assays (luciferase assay or by fluorescent microscope or FACS sorter).

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 Antioxidant 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.

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 Estrogen receptor 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 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 ~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. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

- Nature Reviews Drug Discovery 2013; 12, 931–947
- J Biochem Mol Biol. 2004; 37(2): 139-143.

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.

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Product Category	Product Description (please click category name to see product's pages)
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 lentivirus	Premade shRNA lentivirus for knockdown a specific genes (**P53, LacZ, Luciferase** and more).
microRNA and anti-microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.

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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.