



Pre-made Reporter Lentivirus for Androgen Signal Pathway

Cat#	Product Name	Amounts	
LVP912-P or: LVP912-P-PBS	AR- GFP (Puro) Lentivirus	200ul, ~1 x 10 ⁷ IFU/mL in DMEM medium	
LVP913-P or: LVP913-P-PBS	AR- RFP (Puro) Lentivirus		
LVP914-P or: LVP914-P-PBS	AR- Luc (Puro) Lentivirus		
LVP915-P or: LVP915-P-PBS	AR- Rluc (Puro) Lentivirus		
LVP1240 or: LVP1240-PBS	AR- SEAP (Puro) Lentivirus		
LVP912-B or: LVP912-B-PBS	AR- GFP (Bsd) Lentivirus		
LVP913-B or: LVP913-B-PBS	AR- RFP (Bsd) Lentivirus		
LVP914-B or: LVP914-B-PBS	AR- Luc (Bsd) Lentivirus		
LVP915-B or: LVP915-B-PBS	AR- Rluc (Bsd) Lentivirus		
LVP1241 or: LVP1241-PBS	AR- SEAP (Bsd) Lentivirus		Or
LVP912-N or: LVP912-N-PBS	AR- GFP (Neo) Lentivirus		200ul, ~1 x 10 ⁸ IFU/mL in PBS solution
LVP913-N or: LVP913-N-PBS	AR- RFP (Neo) Lentivirus		
LVP914-N or: LVP914-N-PBS	AR- Luc (Neo) Lentivirus		
LVP915-N or: LVP915-N-PBS	AR- Rluc (Neo) Lentivirus		
LVP1242 or: LVP1242-PBS	AR- SEAP (Neo) Lentivirus		
LVP912-R or: LVP912-R-PBS	AR- GFP (RFP) Lentivirus		
LVP914-R or: LVP914-R-PBS	AR- Luc (RFP) Lentivirus		
LVP915-R or: LVP915-R-PBS	AR- Rluc (RFP) Lentivirus		
LVP1243 or: LVP1243-PBS	AR- SEAP (RFP) Lentivirus		
LVP913-G or: LVP913-G-PBS	AR- RFP (GFP) Lentivirus		
LVP914-G or: LVP914-G-PBS	AR- Luc (GFP) Lentivirus		
LVP915-G or: LVP915-G-PBS	AR- 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.

Androgen signaling pathways:

Androgens, mainly testosterone and 5alpha-dihydrotestosterone (DHT) play significant role in the growth and development of the male reproductive organs. These steroid hormones bring about their biological functions through their associations with Androgen receptor (AR, also known as NR3C4). Activated AR upon ligand binding undergoes conformational change to form a homodimer and interacts tightly with the Androgen Response Element (**A-RE**) which regulates gene expression. Various regulators that regulate androgen induced apoptosis include BRCA1 and Smad3 and Akt, and much more.

GenTarget developed a set of reporting lentivirus to monitor Androgen signaling pathway, the transcriptional activity of the androgen receptor. 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 Androgen Response Element (A-RE) sequence motif (5'-**TGGAGGAACATATTGTATTTATT**).

When androgenic hormones, testosterone or dihydrotestosterone binds to AR, the activated AR binds to ARE which induce the downstream reporter's expression as the result of activation for the minimal promoter. 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.

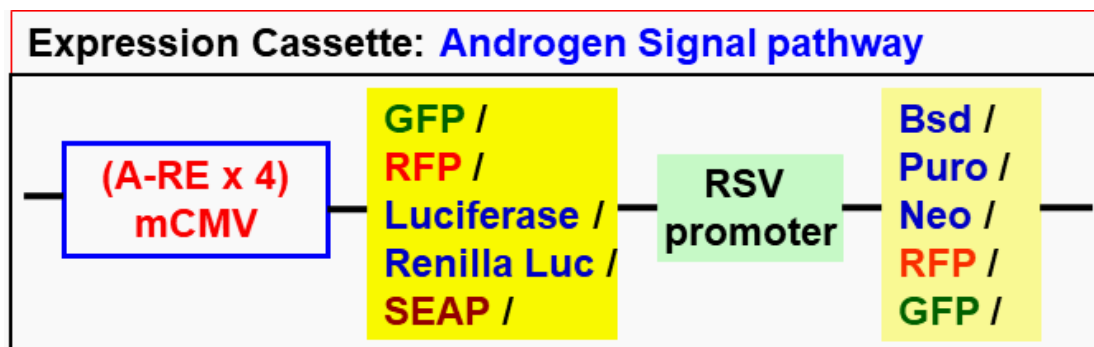


The premade, ready-to-use reporter lentivirus provides an easier, sensitive and quantitative tool to monitor the activity of Androgen signaling pathways in virtually any mammalian cell type. It also allows to generate your own reporting cell line in your desired cell type for study or screen of pathway specific gene-knockdown, over-expression, or chemical / drug/ protein treatment in the cell-based assay.

Premade Androgen Pathway Signaling Lentivirus:

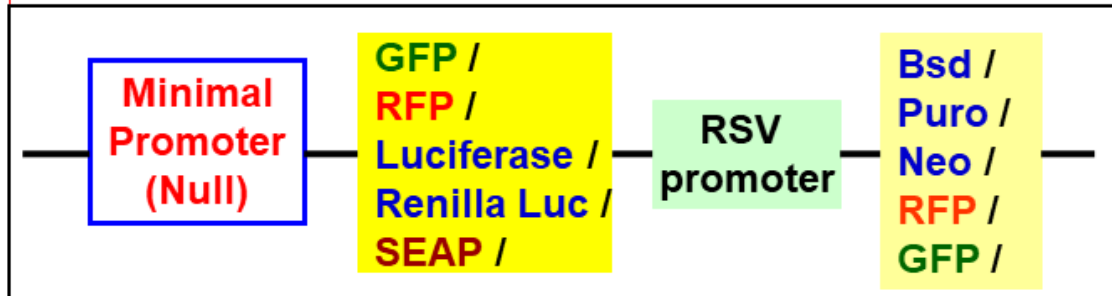
The ready-to-use lentivirus expresses a report (**firefly Luciferases, Renilla luciferase, GFP or RFP**) under mini promoter containing Androgen responsive promoter (**A-RE**). The report is only expressed when the Androgen stimulating signal is present. a different fluorescent selection marker or an antibiotic selection marker under a constitutive RSV promoter, which makes it easier to select the stably infected signal reporting cells (to generate pathway specific sensor cell lines), and provides internal fluorescent normalization reference for virus transduction efficiency (if a fluorescent marker is under the RSV promoter).

A set of **pathway Null Control lentivirus** use the same lentivector backbones except the promoter does not contain any signal pathway's TRE sequences. The control lentivirus are used to set the non-response profile to any pathway stimulus, for specificity of pathway signal response upon stimulation. see lentivector's core structure scheme below.





Lentivector: Pathway Null Controls (CAT#: Path-Ctr)



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 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 Androgen 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.

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.

For more details about premade particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf).

Transduction Protocols:

1. Transduction Protocol for Adhesive cells:

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 μ 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. For stable cell line generation, pass 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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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.
- Return cells to 37°C, CO₂ incubator. Do nothing.

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 48hr~72hr (Depend upon cell type) after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

Day 3 + (optional):



Sort transduced cells by FACS, or select by antibiotic killing. 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).

2. Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO₂ incubator if necessary.

Measure cell density (not grow over 3 million/ml), measured viability should be > 90%. Dilute cells into 1 x 10⁶ cell/ml in complete medium.

Day 1:

- Thaw lentiviral particles at room temperature.
- Add premade lentiviral particles into the diluted cells at a ratio of: 50 to 100 µl virus per 0.5 ml of cells (Note: depending on cell type, you may need to use more or less virus).
- Grow cells in a shaking flask in a CO₂ incubator.

Day 2:

At 24 hours after transduction, add an equal amount of fresh medium containing. Continue growing cells in CO₂ incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Note: Filter wavelength settings:

GFP filter: ~Ex450-490 ~Em525; RFP filter: ~Ex558 ~Em583;

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. 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:

1. Bioessays. 2007 Dec;29(12):1227-38.;
2. The ins and outs of Wingless signaling, Trends Cell Biol. 2004 Jan;14(1):45-53.
3. "Androgen genes". Cell 1992, 69 (7): 1073-1087.
4. Androgen Receptor (AR) Coregulators. Endocrine Reviews 23(2):175-200

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 Products:

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Reporter 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;
CRISPR Gene Editing	Preamde lentivirus express humanized 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



Product Category	Product Description (please click into each category's page)
Infectious Antigens	Lentivirus 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 verified 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-miRNA 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
Sub-cellular Imaging	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.
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



Product Category	Product Description (please click into each category's page)
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviruses 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 fluorescent and antibiotic markers.
CRE, Flp ColorSwitch	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 expressing 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	Premade lentivirus for human and mouse iPS (Myc, NANOG, OCT4, SOX2, FGF4) factors with different fluorescent and antibiotic 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 lentivirus treatment, for validation of the specificity of any 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.