Pre-made Lentiviral Particles for Target Overexpression
(for human, mouse or rat genes / ORFs)

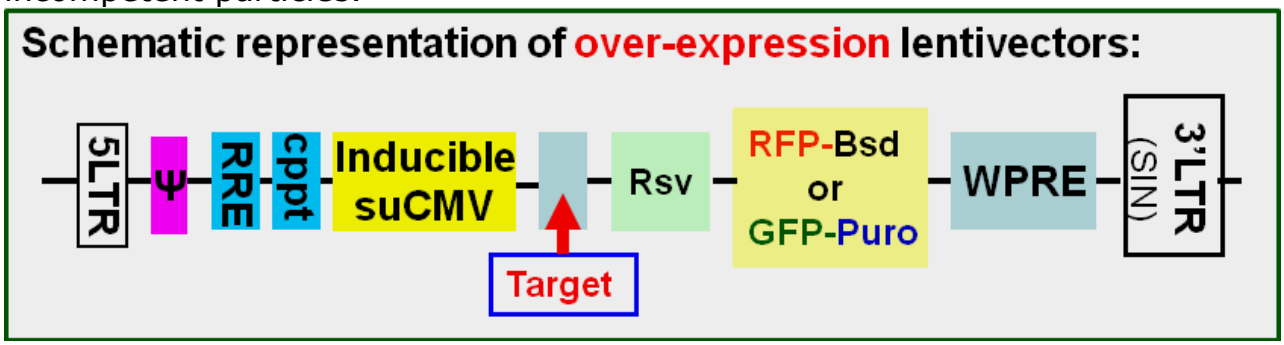
Cat#	GENE Symbol	Gene Name	Alternative Name	NCBI Accession
LVP1123	m Foxo1	forkhead box O1	Afxh; AI876417; FKHR; Fkhr1; Foxo1a	NM_019739.3

Amount: 200ul/vial (1 x 10^7 IFU/ml)
Storage: <-70 °C, avoid repeat freeze/thaw cycles. Stable for 6 months at <-70oC.

Product Description:

The Lentiviral gene delivery system is Human Immunodeficiency Virus-1 (HIV) based lentivector 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 lentivirus a great gene transfer agent.

Pre-made lentiviral particles for specific human or mouse genes are generated from the **optional** inducible lentiviral system (see vector scheme below). The vector used to produce these viral particles includes a self-inactivation feature in its 3' LTR, causing it to only generate replication-incompetent particles.



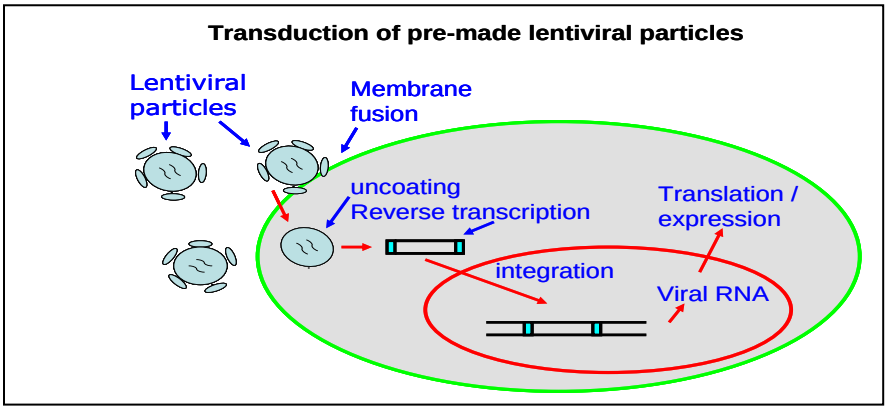
Each particle expresses a fully sequence-verified human, mouse, or rat target matching the CDS sequence in NCBI. The human targets were natively expressed under a tetracycline-inducible suCMV promoter.

Depend on the product, it either a **RFP-Blasticidin** or **GFP-Puromycin** (Fluorescent-antibiotic fusion) dual marker under an RSV promoter allows sorting or selection of transduced cells by RFP or GFP signal, and via Blasticidin or Puromycin killing selection. The fluorescent signal provides a convenient, real-time means to monitor the particles' performance.

All inducible lentiviral particles can be used for regular constitutive expression without need any induction. However it can optionally be used for tetracycline-inducible expression in the presence of the tetracycline repressor protein (TetR). For inducible expression, the target expression is first repressed by TetR, and then induced with the addition of tetracycline. The presence of TetR can be achieved by co-infection with premade TetR lentiviral particles or co-transfection with a TetR expression plasmid, or simply by using a TetR expressing stable cell line. AMSBIO provides TetR lentiviral particles with a variety of antibiotic selection markers for double selection of the target expressing cells.

AMSBIO also provides Negative Control Lentivirus for establishing mock lentivirus treatment in a given cell line. The negative control lentivirus also provides a means to validate the specificity of any target expression effects. The control virus, CAT# CMV-Null-RB or CMV-Null-GP, has a lentivector backbone identical to that of the target expression virus, but expresses only the dual marker.

These ready-to-use particles are packaged in 293T cells and provided as a 200 µl aliquot (Note: the particles can be provided in PBS on special request). Particles are safe and easy to use; simply add them into cultured cells or organs. Each lot of particles is validated and target expression is guaranteed.



Key features:

- High target expression levels driven by extremely strong suCMV promoter
- Easy transduction monitoring that can be readily verified by the RFP signal
- **Optional** tetracycline-inducible expression if desired
- Dual markers: transduced cells can be sorted on the RFP signal or selected by blasticidin resistance
- The lentivirus is ready to use; simply add it into your cell culture **(see transduction carton image above).**
- **Ready to use:** simply add lentivirus into cell culture. No need any other reagents.

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 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, 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 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).

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).

2) Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if necessary.

Measure cell density. When density has reached ~3 x 10^6 cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10^6 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 relevant antibiotics. **Note:** amount of antibiotic depends on cell type. Continue growing cells in CO2 incubator.

Day 3:

At 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Safety Precaution:

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 when handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

References:

1. J Virol. 2000 November; 74(22): 10778–10784.
2. Hum Gene Ther (2003) 14: 1089-105.
3. Mol Ther (2002) 6: 162-8.
4. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).

Warranty:

This product is for research use only. It is warranted to meet its quality as described when used in 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.