GenTarget Inc
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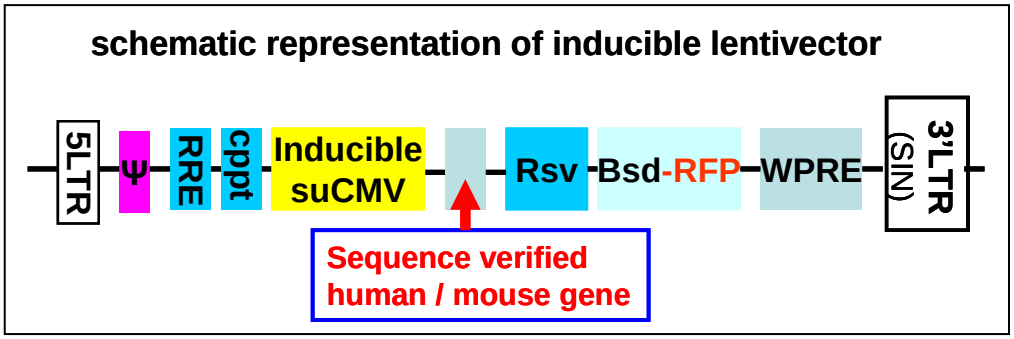
Pre-made Lentiviral Particles for human or mouse gene manual

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

Product Description:

Lentiviral system is a gene delivery tool using lentivector for gene expression or knockdown. Lentivector is HIV-1 (Human Immunodeficiency Virus 1) derived plasmids. It produces lentiviral particles (lentivirus) that are capable to transduce into broad range of mammalian cell types or organs, including primary cells and non-dividing cells both in vivo and in cell culture system, and stably integrated into the transduced cell's genome, independent of cell cycle, for long term expression. Thus lentivirus holds unique promise as gene transfer agents

Pre-made lentiviral particles for specific human or mouse gene are generated from GenTarget's **optional** inducible lentiviral system (see vector scheme below). Vector adapted self-inactivation featured in its 3'LTR, which only generates replication-incompetent particles.



Each particle expresses a full sequence verified human or mouse target, matching to CDS sequence in individual NCBI accession ID (see details in **Product List table** at end of this manual). The human targets were natively expressed under tetracycline inducible **suCMV promoter**. A blasticidin-RFP fusion marker under RSV promoter allows to sort or select transduced cells via RFP signal or via blasticidin antibiotic (**Dual markers**). RFP signal provides a convenient, real-time monitoring the particles performance.

All inducible lentiviral particles can be used as regular constitutive expression. However, when inducible expression is desired, they can **optionally** be used as tetracycline inducible expression in the presence of a repressor protein (tetR, tetracycline regulator protein). As inducible expression, the target expression was first repressed by TetR, and induced after tetracycline added. The presence of TetR can be achieved by co-infected **premade TetR lentiviral particles** or co-trasnfected with a TetR expression plasmid, or simply by a tetR expressing stable cell line. Please see our website for more information about the **inducible lentiviral system**. Gentarget Inc provides the **TetR lentiviral particles** with different antibiotic selection marker for double selection of transduced inducible cells.

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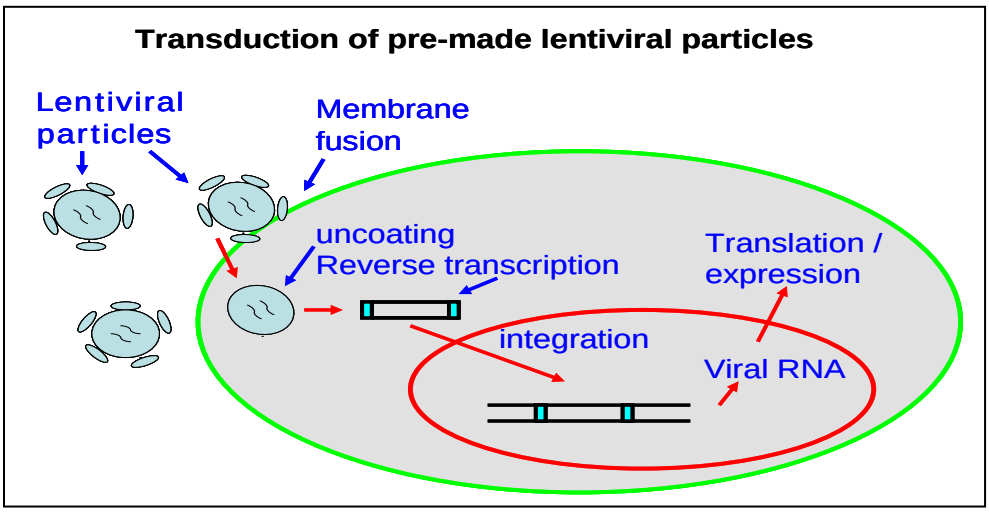
GenTarget also provides the **Negative control lentivirus** (click this link to see each controls) for establishing the control effects of virus infection upon a given cell line, which validates the specificity of any target expression effects.

The ready-to-use particles are packaged in 293T cells and provided as 200ul aliquot without any additives. Particles are safe and easy to use, simply add into cultured cells or organs. Each particles was validated in lot by lot basis and expression is guaranteed. Please see our web-link for "**FAQ about premade lentiviral particles**".



Key features:

1. High target expression level driven by suCMV;
2. High virus titers;
3. **Optional** tetracycline inducible expression;
4. Easy transduction monitoring via the RFP fluorescent signal under microscope;
5. Dual markers: transduced cells can be sorted via a RFP fluorescent signal or selected via blasticidin antibiotic;
6. The lentivirus are ready and easy to use, simply add into your cell culture. **(see transduction carton image above).**

Note:

1. Dependent upon your specific needs, you may design the transduction with different MOI for different levels of expression.
2. For some cell lines, you may add polybrene for transduction enhancement.
3. For general transduction protocols, please refer to out web-link: **Transduction protocols for adhesive and suspension cells.**

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Transduction Protocols:

1. Adhesive cells Transduction Protocols:

Note: A quick transduction protocol is: add 50ul virus into one well in 24-well-plate where cell density is at 50% ~ 75%. At 72 hours after virus added (no need to change medium), visualize the positive rate under fluorescent microscope. For stable cell line generation, pass cell into antibiotic containing medium, or sort the cells via fluorescent signal. Then , select the cells by antibiotics.

Day 0: Seed the desired cells in complete medium at appropriate density incubate overnight. (Note: at the time of transduction, it grows to 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. Add fresh, warmed, complete medium (0.5ml). Thaw the Pre-made lentiviral stock at room temperature. Add appropriate amount of virus stock to obtain the desired MOI. Return cells to 37°C/CO^2 incubator. (Try to avoid thaw and freeze cycles for pre-made lentivirus. But if you cannot use all virus in one time, you still can re-freeze the virus at -80oC for future use. But virus titer will decrease by ~10% for each re-thaw.)

Day 3: At ~72hr after transduction, check the transduction rate *via* fluorescence image with a suitable filter under fluorescent microscope, or calculate the exact transduction rate via Flow Cytometry System (FACS) or any flow cytometry (such as Guava machine).

Day 3 + (optional): Transduced cells can be sorted out via FACS, selected by its specific antibiotics. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literatures about How to generate stable cell lines.)

2. Suspension cells transduction Protocols:

1. Grow your cell in your completed suspension culture medium, shaking in flask in CO^2 incubator if necessary;
2. Measure cell density. When cell grow to ~3 x 10^6 cell/ml, measure cell viability (should be > 90%), then diluted cells into 1 x 10^6 cell/ml in completed medium;
3. Transduction: thaw lentiviral particles at room temperature. Simply add premade lentiviral particle into the diluted cells at ratio of: **50 to 100ul virus per 0.5 ml of cells** (Note: depending on the cell types; you may need to use more or less viruses). Grow cells in flask, shaking in CO2 incubator.
4. At 24 hours after transduction, add equal amount of fresh medium containing related antibiotics (Note: each particles contain an antibiotic marker and the antibiotic amounts to use depends upon cell types). Grow cell in CO^2 incubator.

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5. At 72 hours after transduction, check fluorescence under microscope or calculate the transduction efficiency using cell sorting machine (like FACS or Guava machine).
6. You can sort the fluorescent positive cells, and maintain the antibiotic selection to generate stable cell lines.

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 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. 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.

Related Products:

Product Category	Product Description (please click links below to see product pages)
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
GFP / RFP / YFP / CFP lentivirus	Premade lentivirus expressing a fluorescnet protein (**GFP, RFP, YFP or CFP**) with different antibioic 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.
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
Human and mouse ORFs	Premade lentivirus expressin **hundred of human and mouse ORFs** with RFP-Blastididin fusion dual markers.

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Living cell imging	Pre-made lentivirus particles for Cell Organelle imaging for **Nucleus, Cytoplasm, Endoplasmic Reticulum, Golgi, Mitochondria, Nuclear membrane, Peroxisome, Plasma membrane, Microtubule, Chromatin, Annexin, Actin, Connexin**, and more.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
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.
Negative controls	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.