

Pre-made Lentiviral Particles for target over expression manual (for human, mouse or rat genes ORFs)

Amount: 200ul/vial at $> 1 \times 10^7$ IFU/ml (or in PBS as special request)

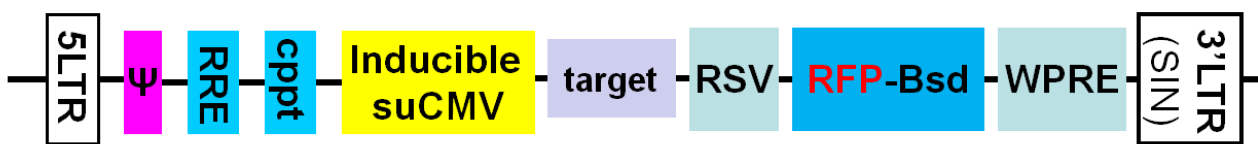
Storage: $<-70^\circ\text{C}$, avoid repeat freeze/thaw cycles. Stable for 6 months at $<-70^\circ\text{C}$.

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 **optional inducible lentiviral system** (see vector scheme below). Vector has adapted self-inactivation feature in its 3' LTR, which only generates replication-incompetent particles.

The representation of optional inducible lentivector core scheme:



Each particle expresses a full sequence verified human, mouse or rat target, matching to CDS sequence of each target according to NCBI accession ID. The human targets were natively expressed under **tetracycline inducible suCMV promoter**. A **blasticidin-RFP** fusion dual marker expressed under RSV promoter allows to sort or to select the transduced cells via RFP signal or via blasticidin antibiotic, respectively. RFP signal provides a convenient, real-time monitoring the particles performance.

All inducible lentiviral particles can be used for 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). For inducible expression, the target expression is first repressed by TetR, and induced after tetracycline addition. The presence of TetR can be achieved by co-infection of **premade TetR lentiviral particles** or co-transfected with a TetR expression plasmid, or simply by a tetR expressing stable cell line. Please see our website for more

AMSBIO | www.amsbio.com | info@amsbio.com

 **UK & Rest of the World**
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

 **North America**
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

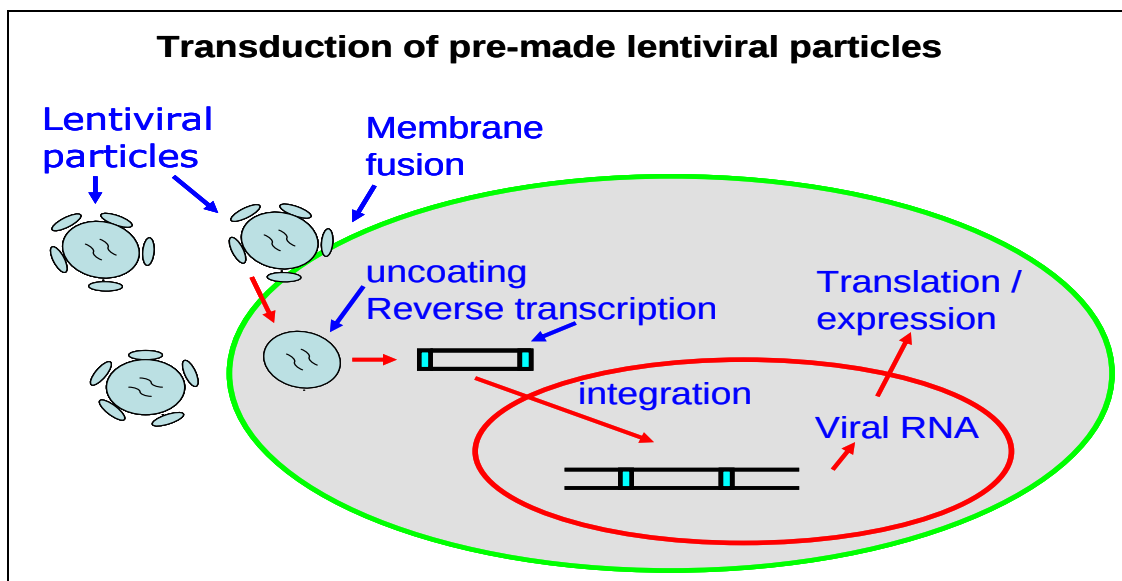
 **Germany**
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

 **Switzerland**
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

information about the **inducible lentiviral system**. Amsbio provides the **TetR lentiviral particles** with different antibiotic selection marker for double selection of the inducible target expression cells.

We also provide the Negative control lentivirus for establishing controls for lentivirus treatment in a given cell line, which also validates the specificity of any target expression effects. The control virus to use is: **CMV-Null-RB** which has the identical lentivector backbone as target expression virus, but does not express any target except the same dual marker.

The ready-to-use particles are packaged in 293T cells and provided as 200ul aliquot (Note: the particles can be provided in PBS as special request). Particles are safe and easy to use, simply add into cultured cells or organs. Each particle is validated in lot by lot basis and the target expression is guaranteed.



Key features:

1. High target expression level driven by strong suCMV promoter;
2. High virus titers that can be readily verified by the RFP fluorescent signal;
3. **Optional** tetracycline inducible expression when desirable;
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 is ready and easy to use, simply add into your cell culture. (see transduction carton image above).

AMSBIO | www.amsbio.com | info@amsbio.com

 **UK & Rest of the World**
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

 **North America**
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

 **Germany**
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

 **Switzerland**
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Note:

1. Depending on 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.

Transduction Protocols:

1. Adhesive cells Transduction Protocols:

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

Day 0: Seed the desired cells in complete medium at 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: Remove the culture medium. Add fresh, warm, 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/CO₂ incubator. (Try to avoid thaw / freeze cycles for pre-made lentivirus. But if you cannot use all virus at once, you still can re-freeze the virus at -80°C 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* fluorescent imaging 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 via FACS and selected by its specific antibiotic. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line. (Refer to any literature on How to generate stable cell lines.)

2. Suspension cells transduction Protocols:

1. Grow your cell in complete suspension culture medium, shaking flask in CO₂ incubator if necessary;
2. Measure cell density. When cell grow to $\sim 3 \times 10^6$ cell/ml, measure cell viability (should be > 90%), then dilute cells into 1×10^6 cell/ml in complete medium;

AMSBIO | www.amsbio.com | info@amsbio.com

 **UK & Rest of the World**
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482

 **North America**
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218

 **Germany**
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880

 **Switzerland**
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

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 type, you may need to use more or less virus). Grow cells in flask, shaking in CO₂ incubator.
4. At 24 hours after transduction, add equal amount of fresh medium containing related antibiotic (Note: each particles contain an antibiotic marker and the antibiotic amounts to use depends upon cell types). Grow cell in CO₂ incubator.
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:

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. Wear 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.
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.

Those products are provided for research use only!

AMSBIO | www.amsbio.com | info@amsbio.com



UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482



North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218



Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880



Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85

Related Products:

Product Category	Product Description (please Category name to see product's pages)
Fluorescent protein.	Premade Lentivirus for GFP/ CFP/ YFP/ RFP
Human and mouse ORFs.	Premade lentivirus expressing human and mouse ORFs with RFP-Blasticidin fusion dual markers.
CRE recombinase.	Premade lentivirus 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 expressing 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 antibiotic markers
T-antigen Expression	Expression of large and small T antigen with different selection markers
Cell Organelle imaging.	Premade lentivirus for cell organelle imaging. The fluorescent marker GFP/RFP/CFP is localized in different cell organelles for live cell imaging.
LacZ expression	Expression of full length β-galactosidase (lacZ) with different selection markers
Fluorescent-ORF fusion.	Pre-made lentivirus for expression of " GFP/RFP/CFP-ORF " fusion targets.
Pre-made shRNA lentivirus.	Premade shRNA lentivirus for knockdown of a specific gene (P53, LacZ, Luciferase and more).
microRNA and anti-microRNA lentivirus.	Premade lentivirus expressing human or mouse precursor miRNA and anti-miRNA lentivector and virus for human and mouse miRNA.
Negative control lentiviruses.	Premade negative control lentivirus with different markers : serves as a 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.

AMSBIO | www.amsbio.com | info@amsbio.com



UK & Rest of the World
184 Park Drive, Milton Park
Abingdon OX14 4SE, UK
T: +44 (0)1235 828 200
F: +44 (0) 1235 820 482



North America
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
T: +1 (800) 987-0985
F: +1 (617) 945-8218



Germany
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
T: +49 (0) 69 779099
F: +49 (0) 69 13376880



Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
T: +41(0) 91 604 55 22
F: +41(0) 91 605 17 85