

Product Information and Protocols (V2.2)

Product Name: Ready-To-Use Lentivirus Marker Supernatant: Lenti-Red-Blasticidin

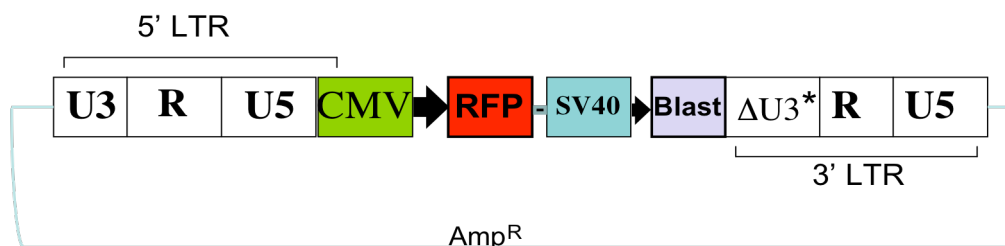
Catalog ID: LG502-b

Description: This lentiviral supernatant was produced by co-transfecting 293T cells with a lentiviral vector containing RFP (red fluorescence protein) and anti-blasticidin genes, with two other plasmids which make the lentiviral envelop proteins and VSV-G protein. The viral supernatant was aliquot and stored at -70°C immediately after purification and concentration.

Features & Advantages:

1. Ready-to-use, just add it to cells. That's it!
2. Cells are permanent labeled.
3. No lentivirus expertise or troublesome cloning required.
4. Far fewer concerns on biosafety issues.
5. Get stable cell lines within three days by FACS.
6. Applicable *in vivo* for transgenic animals.
7. Genes of interest constructed into the latest version (self-inactivating) lentiviral vector.
8. Viral particles produced in specified packaging cells with optimized protocols.
9. Viral supernatants collected and purified to eliminate potential toxic materials.
10. Lenti-markers titers $\geq 10^7$ viral particles/ml, marked with true fluorescence protein expression (NOT by PCR detection).
11. Further selection of positive cells with antibiotics (Blasticidin).

Functional Map of Lenti-Red-Blasticidin^R



ΔU3* contains only 45 nt of the U3 region of lentiviral 3' long terminal repeat (LTR), making it impossible for the virus to replicate itself called "self-inactivated"

Titration: The titer of Read-to-use lentivirus supernatant, determined by FACS, or using a fluorescence microscope, is approximately containing 1×10^7 lentiviral Transducing particles per ml. The RFP expression was tested on 293T cells on the 3rd day after viral transduction. The titer was calculated by comparison with a standard virus product.

Components/Reagents: Ready-to-use supernatant is frozen stock in Dulbecco’s Modified Eagle’s Medium with 10% heat-inactivated fetal bovine serum and penicillin-streptomycin (Custom “ready to use” virus supernatant can also be made in “serum-free” medium).

Storage & Stability: Store this product in -80°C freezer upon receiving it. Ready-to-use viral supernatant is very sensitive to freeze/thaw cycle, only a necessary amount should be thawed every time to ensure the titer of virus. If stored properly, the viral stocks should be suitable for use for up to 6 months. After long-term storage, it is recommended the viral stocks be re-titrated before use.

Additional Notes:

1. Polybrene. Polybrene is a small, positively charged molecule that binds to cell surfaces and neutralizes surface charge and greatly enhances transduction by lentiviruses. Polybrene can be added to the viral dilution before adding the virus to the cells. However the amount added must dilute out to **8ug/ml** in the culture medium after transduction. Cells vary in the amount of polybrene that they will tolerate. Typically it is between 1 to 10 µg per ml. Polybrene can be toxic to terminally differentiated neurons and dendritic cells.
2. Multiplicity of Infection (MOI). Cells cell type/species suggested MOI range Polybrene (Data from Invitrogen).

<u>Cell Type</u>	<u>M.O.I.</u>	<u>Polybrene Concentration/ml</u>
HT1080 human fibrosarcoma	1 to 10	6 ug/ml
293 human embryonic kidney	5 to 10	3 ug/ml
HeLa human epithelial carcinoma	5 to 10	3 ug/ml
Rat cortical neurons	10 to 25	none
Rat neural stem cells	10 to 100	none

3. Fact on Lenti-Red.
Lenti-Red: Excitation: 553 nm; Emission: 575 ± 15 nm
4. Safety issues: In the US, the CDC suggests use of Lentiviral stocks to be treated as Biosafety Level 2 organisms. For further information on BL-2 guidelines and lentivirus handling, please refer to: “Biosafety in Microbiological and Biomedical Laboratories”, 4th Edition and Centers for Disease Control and their website: www.cdc.gov. This 3rd generation HIV-based lentiviral vector product has enhanced biosafety and minimized relation to the wild-type human HIV-1 virus. Since it’s “self-inactivated”, the chance for viral revival is minimal.
5. Precautions and Disclaimer. These products are for R&D use only, not for drug, household, or other uses. Though the lentiviral transduction particles produced are replication incompetent, it is highly recommended that they be treated as Risk Group Level 2 (RGL-2) organisms. Follow all published RGL-2 guidelines for handing and waste decontamination. Also, use extra caution when using lentiviral transduction particles that express RFP or other molecules.

Protocol for Suspension Cells

Various protocols have been used for transducing suspension cells. We recommend transducing suspension cells with our Ready-to-Use lentivirus in a small volume and gently centrifuging the cells through the supernatant. Here we give an example of transducing cells by centrifugation in a 6-well plate, cells in tissue culture flasks can be harvested in a 15ml conical tube and follow the same procedures.

1. Day 1: Plate the mammalian cell line of choice in complete medium 24 hours prior to transduction at 5×10^5 /ml in a well of a 6-well plate (use 2 ml/well).
2. Day 2: Thaw the lentiviral stock at room temperature. Add lentiviral supernatant at 1:1 ratio of cell culture medium.

Note: You **Do Not** need to add polybrene for viral transduction. Our products already contain the appropriate concentration of Polybrene for a 1:1 dilution in the cell culture medium. If you use a different ratio, such as 1:10, you need to add more polybrene accordingly (we recommend a final conc. of 8 μ g/ml).

- a. When transducing a lentiviral construct into a cell line for the first time, it is recommended that a range of MOIs (such as 0, 1, 10 and 50) be used to determine the optimal degree of mark gene expression.
 - b. When overnight incubation presents a toxicity concern, cells may be incubated for as short as 4 hours before changing the medium.
 - c. Spin the plate at 3000 x g for 4 hrs at 30°C (Please note: This procedure is necessary only for suspension cells). After spin, take out medium containing viruses and re-suspend cells in fresh complete medium. Return cells to 37°C/CO₂ incubator for 12 hrs or overnight. The same procedure can be repeated to increase transduction rate.
3. Day 3: Remove the old medium and replace it with fresh, complete culture medium and keep to incubate overnight.
 4. Day 4: Check fluorescence which will be detectable with an appropriate wavelength under fluorescent Microscope.

Protocol for Adherent Cells

1. Day 1: Plate the mammalian cell line of choice in complete medium 24 hours prior to transduction at 5×10^5 /ml in a well of a 6-well plate (use 2 ml/well).
2. Day 2: Thaw the lentiviral stock at room temperature and dilute (if necessary) the virus to a suitable MOI (multiplicity of infection) with fresh complete medium. (The volume of stock viral supernatant can be 1:10 to 1:1 of the volume of medium that will be used to culture the target cells). Remove the culture medium from the cells. Add fresh medium containing virus.

Note: You **Do Not** need to add polybrene for cell transduction. Our products already contain the appropriate concentration of Polybrene for a 1:1 dilution in the cell culture medium. If you use a different ratio, such as 1:10, you need to add more polybrene accordingly (we recommend a final conc. of 8 μ g/ml).

3. Day 3: Remove the medium containing virus and replace with fresh and complete culture medium.
4. Day 4: Check fluorescence which will be detectable with an appropriate wavelength under fluorescent microscope.

References

1. Zufferey, R., et al., Multiply attenuated lentiviral vector achieves efficient gene delivery in vivo. *Nat. Biotechnol.* **15**, 871-85 (1997).



2. Zufferey, R., et al., Self-inactivating lentivirus vector for safe and efficient in vivo gene delivery. *J Virol.* **72**, 9873-80 (1998).

3. Burns, J.C., et al., Vesicular Stomatitis Virus G Glycoprotein Pseudotyped Retroviral Vectors: Concentration to a Very High Titer and Efficient Gene Transfer into Mammalian and Nonmammalian Cells. *Proc. Natl. Acad. Sci. USA*, **90**, 8033-8037 (1993).

4. NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) 2002 (<http://www4.od.nih.gov/oba>) Polybrene is a registered trademark of Abbott Laboratories Corporation.