



Lenvirus for near-infrared RFP (niRFP) fluorescent marker

| Catalog# | Product Name | Amounts |
|----------------------------|---|----------------------------------|
| LVP558 | niRFP (CMV, Puro) Lentiviral particles | 1x10 ⁷ IFU/ml x 200ul |
| LVP559 | niRFP (CMV, Bsd) Lentiviral particles | 1x10 ⁷ IFU/ml x 200ul |
| LVP560 | niRFP (CMV, Neo) Lentiviral particles | 1x10 ⁷ IFU/ml x 200ul |
| LVP561 | niRFP (EF1a, Puro) Lentiviral particles | 1x10 ⁷ IFU/ml x 200ul |
| LVP562 | niRFP (EF1a, Bsd) Lentiviral particles | 1x10 ⁷ IFU/ml x 200ul |
| LVP563 | niRFP (EF1a, Neo) Lentiviral particles | 1x10 ⁷ IFU/ml x 200ul |
| LVP558-PBS | niRFP (CMV, Puro) Lentivirus in PBS | 1x10 ⁸ IFU/ml x 200ul |
| LVP559-PBS | niRFP (CMV, Bsd) Lentivirus in PBS | 1x10 ⁸ IFU/ml x 200ul |
| LVP560-PBS | niRFP (CMV, Neo) Lentivirus in PBS | 1x10 ⁸ IFU/ml x 200ul |
| LVP561-PBS | niRFP (EF1a, Puro) Lentivirus particles | 1x10 ⁸ IFU/ml x 200ul |
| LVP562-PBS | niRFP (EF1a, Bsd) Lentivirus in PBS | 1x10 ⁸ IFU/ml x 200ul |
| LVP563-PBS | niRFP (EF1a, Neo) Lentivirus in PBS | 1x10 ⁸ IFU/ml x 200ul |

Storage: < -70 °C, avoid repeat freeze/thaw cycles. Stable for > 6 months.

Product Description:

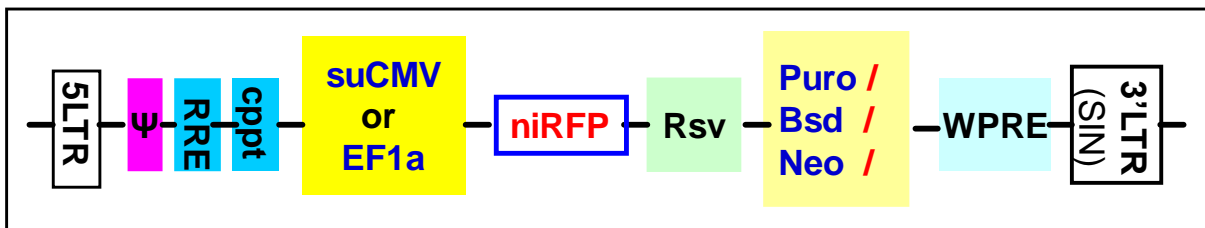
Lentiviral particles or lentivirus is a gene delivery tool produced from lentivectors for gene expression or knockdown. GenTarget's lentivector system is Human Immunodeficiency Virus-1 (HIV) based plasmids for gene expression and knockdown. The lentivectors are used to generate lentiviral particles (lentivirus) 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.

The near-infrared Red fluorescent protein (niRFP) was characterized with high brightness and photostability, with the far-red part of the spectrum, which makes it an excellent fluorescent marker. It can be used for whole-body images with better contrast and brighter images than other fluorescent proteins [3]. Beware you may need to supplement your cells with biliverdin (biliverdin IXa) for maximal brightness.



GenTarget constructs a set of **niRFP** expression lentivirus under either enhanced CMV promoter, or the enhanced EF1a promoter, containing different antibiotic selection (Puromycin, Blasticidin, or Neomycin). The suCMV promoter demonstrates the highest expression level in the majority of cell types. The engineered EF1a promoter is non-tissue specific, highly expressed in all cell types, and less likely to be silenced after long-term culture. See the core lentivector structure scheme below:

Schematic representation of **niRFP** expression lentivectors:



Lentivirus are pseudotyped with VSVG, are provided in two formats:

- Regular particles in DMEM medium containing 10% FBS and 60 µg/ml polybrene (10 x stock).
- Particles concentrated and buffer exchanged into PBS for used in the hard to transduced cell types or for *in vivo* application.

For general questions about our ready-to-use lentiviral particles, please see [FAQ for pre-made lentiviral particles](#) (.pdf) on our website. (<http://www.gentarget.com/pdf/FAQ-Premade-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 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 $0.5 \times 10^5/\text{ml} \times 0.5\text{ml}$ 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, CO₂ 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 cytometer (FACS or Guava).

Day 3 + (optional):

Sort transduced cells by FACS, and select for antibiotic resistance.

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. When density has reached $\sim 3 \times 10^6$ cells/ml, measured viability should be > 90%. Dilute cells into 1×10^6 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.

Note: Filter wavelength settings:

niRFP filter: Ex:670~690 nm; Em: 713 ~ 770nm;

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. Nature Biotechnology volume 29, pages757-761(2011).

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: GenTarget's Pre-made lentivirus Products:

| Lentivirus Category (click to see) | Product Description |
|--|--|
| Target Expression | Premade lentivirus express a human, mouse or rat gene with Fluorescent-Antibiotic fusion dual selection. |
| Luciferase expression | Premade lentivirus express all kinds of luciferase: firefly; Renilla; Cypridina; Red-Luc; Nano-Luc , with different fluorescent and antibiotic selection. |
| Fluorescent markers | Preamde lentivirus express human codon optimized fluorescent protein, GFP / RFP / CFP / BFP / YFP/niRFP /unstable GFP, etc. |
| Cytoskeleton Imaging | Fluorescent (GFP / RFP/ CFP) labelled cell skeleton protein (Actin; Tubulin; Paxillin; Vimentin) |



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| Cell Organelle imaging | Premade lentivirus for cell organelle imaging. The fluorescent labelled cell organelle lentivirus for living cell imaging. |
| CRISPR /hu CAS9 | Preamde lentivirus express humanized wild-type Cas9 endonuclease for genomic editing by CRISPR |
| Fluorescent Fusion target | Lentivirus express the " Fluorescent-Target " fusion proteins. A desired target is fused to Green, Blue, Red, or Cyan Fluorescent Protein, demonstrating the target's functionality and localization |
| CRE recombinase | Premade lentivirus for expressing nuclear permeant CRE recombinase with different fluorescent and antibiotic markers. |
| LoxP ColorSwitch | Premade lentivirus expressing "LoxP- GFP -Stop-LoxP- RFP " cassette, used to monitor the CRE recombination event in vivo. |
| SEAP Reporter | SEAP (Secreted Embryonic Alkaline Phosphatase) secreted expression lentivirus under different promoter. |
| TetR repressor expression | Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system. |
| rtTA Expression | Lentivirus express the reverse tetracycline transcription activator gene, rtTA-M2 with different selection. |
| Pathway Reporter | Different Report lentivirus (Luc, RFP, GFP, SEAP) under a pathway specific response promoter. |
| Cell Immortalization | Comprehensive lentivirus for cell immortalization, for different cell types. |
| Cell Specific reporter | Different Report lentivirus driven by cell specific promoter. |
| Infectious Antigens | Lentivirus express all kinds of infectious antigens. |
| Viral Like Particle (VLP) | Lentiviral particles pseudo-typed with high density of surface envelope protein. |
| Immuno Therapy | Lentivirus products for Immuno Therapy application. |
| 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 |
| Anti-miRNA | Pre-made lentivirus expression a specific anti-miRNA |



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| lentivirus | cassette. |
| Pre-made 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 control lentiviruses | Premade negative control lentivirus with different markers : serves as the negative control of lentiviruses treatment, for validation of the specificity of any lentivirus target expression effects. |
| Other Enzyme | Ready-to-use lentivirus, expressing specific enzymes with different selection markers. |