



Lentivirus express COVID-19 S protein and its mutants

| Cat# | Product Name | Amount |
|-----------------------------|--|-------------------------------------|
| LVP1329-PBS | COVID-19 S Protein (6His) Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1330-PBS | COVID-19 S1-RBD (6His) Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1433-PBS | COVID-19 S Protein Mutant (K417T, E484K, N501Y)-6His Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1434-PBS | COVID-19 S Protein Mutant (L452R)-6His Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1435-PBS | COVID-19 S Protein Mutant (W152C, L452R, D614G)-6His Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1436-PBS | COVID-19 S Protein Mutant (S477N)-6His Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1437-PBS | COVID-19 S Protein Mutant (L452R, D614G)-6His Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1438-PBS | COVID-19 S Protein Mutant (K417N, E484K, N501Y)-6His Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |
| LVP1439-PBS | COVID-19 S Protein Delta (L452R, T478K)-6His Expression Lentivirus | 200 ul x (1x10 ⁸ IFU/ml) |

Amount: 200ul/vial (1 x 10⁸ IFU/ml)

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

About Lentivirus (Lentiviral Particles):

GenTarget's 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.



What is COVID-19 S protein (SARS-CoV-2 Spike protein) and its mutants:

The COVID-19 pandemic emerged as a severe threat to human health. It is caused by coronavirus 2 virus (SARS-CoV-2). Coronaviruses are positive-stranded RNA viruses, featuring the largest viral RNA genomes known to date (27-31 kb). The coronavirus surface presents a trimetric S protein consisting of N-terminal S1 and C-terminal S2. The S1 consist of N-terminal domain (NTD) and the receptor binding domain (RBD). SARS-CoV-2 virus attaches human cells via human ACE2 receptor and the serine protease, TMPRSS2, through its Spike (S) Protein. The RBD is responsible for binding to host cell surface ACE2, the S2 for binding of host protease TMPRSS2.

Covid-19 virus constantly changes through mutation. The mutations occur in Spike protein let virus to escape human immune-system, reduce neutralization by antibodies generated against wild-type virus or vaccination.

Why Spike proteins is the drug development target?

Spike (S)-glycoprotein of the virus interacts with a cellular receptor and mediates membrane fusion to allow viral entry into susceptible target cells. To stop SARS-CoV-2 infection, one strategy is to block the virus entry human cells, i.e to interrupt the binding between virus and human receptor ACE2 or TMPRSS2. Researchers try to raise antibodies targeting at S1 protein or at RBD epitopes. Those antibodies prevent virus from binding to human ACE2. Therefore, S protein is the target for vaccines, therapeutic antibodies, and diagnostics.

Identifying the S-protein mutants and testing the vaccine efficacy on COVID-19 mutants (different variants) will greatly help to stop the virus spread. The new vaccines can be developed again the mutants.

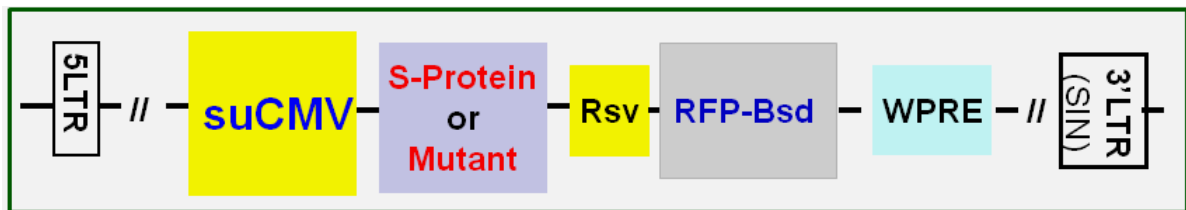
Expression lentivirus for S1 protein or its mutants:

GenTarget developed lentivirus products that express **S protein** and **its mutants**, carrying a poly-histidine tag at the C-terminus (**6-His tag**). Simply add those ready-to-use lentivirus into any desired mammalian cells, your cells will express the entire S protein, or its mutant, and you can use the cells for any cell based assay or detection. You can also purify the mammalian expressed S protein or mutant via Ni NTA affinity column (6His), and use them as antigens in antibody development or validation, ELISA plate detection and so on.



Product Features:

The target was expressed under the enhanced CMV promoter for highest expression level in common used mammalian cells, like CHO, 293FT-suspension cells. A **RFP-Blasticidin** (Fluorescent-antibiotic fusion) dual marker under an RSV promoter allows sorting or selection of transduced cells by Red Fluorescent signal, and via Blasticidin killing selection when you need to generate the expression cell line. The fluorescent signal provides a convenient, real-time means to monitor the particles' performance. See the lentivector core expression scheme below:



These ready-to-use, concentrated lentivirus are packaged in 293T cells and provided as a 200 μ l aliquot in PBS solution. Lentivirus are safe and easy to use. Simply add them into cultured cells, 3 days later you can select the transduced cells, and grow up to expend the target expression, and purify the S-protein when desired.

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 μ l 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 0.5×10^5 /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. Or simply add 50ul per 0.5 ml culture (1:10 dilution).
- 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 72 hours after transduction, check fluorescence with a fluorescence microscope or calculate the transduction efficiency using a cell sorter such as FACS or Guava if desired. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line if desired.

Otherwise, Expend the cell culture under blasticidin containing medium to achieve your desired expression scale for later purification. The expressed protein can be purified via Ni NTA affinity column (6His-tag).

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 μ l 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 if desired. Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line if desired.

Otherwise, Expend the cell culture under blasticidin containing medium to achieve your desired expression scale for later purification. The expressed protein can be purified via Ni NTA affinity column (6His-tag).

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.

Attachment: GenTarget's Pre-made lentivirus Products:

| Lentivirus Category (click to see) | Product Description |
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| 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. |



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| 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) |
| 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 | Premde 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 |



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| | different selection markers |
| Anti-miNA lentivirus | Pre-made lentivirus expression a specific anti-miRNA 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. |