GenTarget Inc

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Lentivirus express COVID-19 S protein and its mutants

Cat#	Product Name	Amount
LVP1329-PBS	COVID-19 Spike Protein (6His) Expression Lentivirus	200 ul x ($1x10^8$ IFU/ml)
LVP1330-PBS	COVID-19 S1-RBD Domain (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 (1×10^8 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.

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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.

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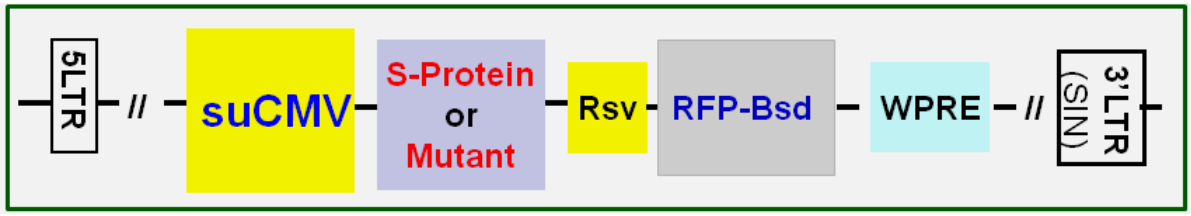
Product Features:

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 µl 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 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 x 10^5/ml x 0.5ml in a well of a 24-well plate.

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Day 1:

- 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, CO_2 incubator. Do nothing.
  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 48hr~72hr (Depend upon cell type) after transduction, check the transduction rate by fluorescence microscopy or calculate the exact transduction rate by flow cytometry (FACS or Guava).

Day 3 + (optional):

Sort transduced cells by FACS, or select by antibiotic killing. A pilot experiment should be done to determine the antibiotic's kill curve for your specific cell line (refer to the pertinent literature on generation of stable cell lines).

2. Transduction Protocol for Suspension Cells:

Grow cells in complete suspension culture medium; use a shaking flask in a CO^2 incubator if required.

Measure cell density (not grow over 3 million/ml), measured viability should be > 90%. Dilute cells into 1 x 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. Continue growing cells in CO2 incubator.

Day 3+:

At 48 hour to 72 hours (Depend upon cell type) after transduction, check fluorescence with a fluorescence microscope or calculate the

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transduction efficiency using a cell sorter such as FACS or Guava. Pass cells into 0.5 million/ml density in completed medium containing the corresponding antibiotic (**Note:** amount of antibiotic depends on cell type. A killing curve must pre-established). Sort for fluorescence positive cells and maintain antibiotic selection to generate a stable cell line.

Note: Filter wavelength settings:
RFP filter: ~Ex558 ~Em583;

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 product categories.

Product Category	Product Description (please click into each category's page)
Pathway Reporter	Repoter Lentivirus for all kinds of pathway screening assays
Cell Immortalization	Lentivirus for cell immortalization: Large T-antigen, hTERT, EBNA1/EBNA2, HpV16-E6/E7, Adenovial E1A, Kras_G12V, HOXA9, et al.

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Product Category	Product Description (please click into each category's page)
ImmunoOncology Research	Lentivirus products for immuno therapy research: CAR and TCR; Assay Cell Lines for T-cell targeted killing assay and other cell-based assays; over-expression lentivirus products for the immune response targets; Cell surface antigens (CDs); immune checkpoint / Receptors; CRISPR gene Repair and knock-IN lentivirus; CRISPR knockout lentivirus;
CAR-T, TCR Lentivirus	**CARs** Lentivirus: Anti-CD19 /CD20 /CD22 /BCMA /hHER2 /HLA-A2 /TGFβ; **TCRs**: MART-1/ NY-ESO1/ CD1d-α-GalCer/ TRαV3-F2A-TRβV5-6;
CRISPR Gene Editing	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease, the **dCas9**, gRNAs, **CRISPR** gene editing research
Epigenomic: CRISPRi and CRISPRa	"**dCas9-Protein**" fusion Lentivirus for epigenomic modification, resulted in CRISPR interference (CRISPRi) or activation (CRISPRa).
Cell-Specific Reporter	a set of reporter lentiviruses to express a luminescence or fluorescent reporter (firefly Luciferase, Renilla luciferase, RFP or GFP fluorescent marker) under a tissue specific promoter
Infectious Antigens	Llentivirus that express all kinds of infectious antigens with C-term 6His-tag.
Virus Like Particles (VLP)	Lentiviral Like Particles, pseudo-typed with a different envelope proteins.
Non-integrating LV	Integration Defective Lentivirus, express different targets for transient expression without the unwanted insertional mutagenesis.
shRNA Knockdown	Knockdown verifeid and customized shRNA lentivirus for target knockdown,
microRNA lentivirus	Premade lentivirus expression human or mouse **precursor miRNA**. And **anti-miRNA** lentivector and virus for human and mouse miRNA.
Anti-miNA lentivirus	Pre-made lentivirus expression a specific anti-miRNA cassette.
Human and mouse ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.

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Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla, Red-Luc and more,** with different antibiotic selection markers.
Fluorescent Markers	Lentivirus express all commonly used fluorescent proteins: GFP, RFP, CFP, BFP YFP, niRFP, unstable GFP and others.
Luminescent Imaging	Lentivirus express Nano-Latern as Bio-probes for in vivo imaging of sub-cellular structural organization and dynamic processes in living cells and organisms
Sub-cellular Imaging	Lentivirus contain a well-defined organelle targeting signal fusioned to a fluorescent protein, great tools for live-cell imaging and for dynamic investigation of sub-cellular signal pathways.
Cytoskeleton Imaging	A fluorescent marker (GFP, RFP or CFP) fusion with a cellular structure protein, provides a convenient tool for visualization of cytoskeletal structure
Unstable GFP	Lentivirus express the the destabilized GFP (uGFP) which provides fast turnover responses in signal pathway assay and in knockdown / knockout detection
near-infrared RFP	The near-infrared Red fluorescent (niRFP) expression Lentiviurs provides the whole-body images with better contrast and brighter images
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.
CRE, Flp ColorSwtich	Lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" or "FRT-GFP-Stop-FRT-RFP" cassette, used to monitor the CRE or Flp recombination event in vivo.
SEAP Reporter	lentivirus expressing SEAP under different promoters (TetCMV, EF1a, CAG, Ubc, mPGK, Actin-beta or a signal pathway responsive promoter),
TetR Repressor	Premade lentivirus expressin TetR (tetracycline regulator) protein, the repressor protein for the inducible expression system.
rtTA Expression	rtTA binds to the tetracycline operator element (TetO) in the presence of doxycycline (Dox). Used for Tet-On /OFF

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Product Category	Product Description (please click into each category's page)
	inducible system.
iPS factors	Premde lentivirus for human and mouse iPS **(Myc, NANOG, OCT4, SOX2, FLF4**) factors with different fluorescent and antibitoic markers
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Negative control lentiviruses	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.
Other Enzyme expression	Ready-to-use lentivirus, expressing a specific enzymes with different selection markers.
Ultra titer lentivirus	Ultra-titer lentivirus used for the hard-to-transduced cells and for in vivo manipulation of sperm cells, or stem cells.

