GenTarget Inc

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Pre-made Negative Control Lentiviral Expression Particles

Cat#	Product Name	Amounts
CMV-Null-Bsd	CMV Control lentiviral particles (Bsd)	**200ul/each**, ~1 x 10^7 IFU/mL in DMEM containing 10% FBS,
CMV-Null-Neo	CMV Control lentiviral particles (Neo)	
CMV-Null-Puro	CMV Control lentiviral particles (Puro)	
CMV-Null-GB	CMV Control lentiviral particles (GFP-Bsd)	
CMV-Null-GP	CMV Control lentiviral particles (GFP-Puro)	
CMV-Null-RP	CMV Control lentiviral particles (RFP-Puro)	
CMV-Null-RB	CMV Control lentiviral particles (RFP-Bsd)	
EF1a-Null-Bsd	EF1a Control lentiviral particles (Bsd)	
EF1a-Null-Neo	EF1a Control lentiviral particles (Neo)	
EF1a-Null-Puro	EF1a Control lentiviral particles (Puro)	
EF1a-Null-GB	EF1a Control lentiviral particles (GFP-Bsd)	
EF1a-Null-GP	EF1a Control lentiviral particles (GFP-Puro)	
EF1a-Null-RP	EF1a Control lentiviral particles (RFP-Puro)	
EF1a-Null-RB	EF1a Control lentiviral particles (RFP-Bsd)	
CMV-Null-Bsd-PBS	CMV Control lentiviral particles (Bsd) in PBS	**200ul/each**, ~1 x 10^8 IFU/mL in PBS solution
CMV-Null-Neo-PBS	CMV Control lentiviral particles (Neo) in PBS	
CMV-Null-Puro-PBS	CMV Control lentiviral particles (Puro) in PBS	
CMV-Null-GB-PBS	CMV Control lentiviral particles (GFP-Bsd) in PBS	
CMV-Null-GP-PBS	CMV Control lentiviral particles (GFP-Puro) in PBS	
CMV-Null-RP-PBS	CMV Control lentiviral particles (RFP-Puro) in PBS	
CMV-Null-RB-PBS	CMV Control lentiviral particles (RFP-Bsd) in PBS	
EF1a-Null-Bsd-PBS	EF1a Control lentiviral particles (Bsd) in PBS	
EF1a-Null-Neo-PBS	EF1a Control lentiviral particles (Neo) in PBS	
EF1a-Null-Puro-PBS	EF1a Control lentiviral particles (Puro) in PBS	

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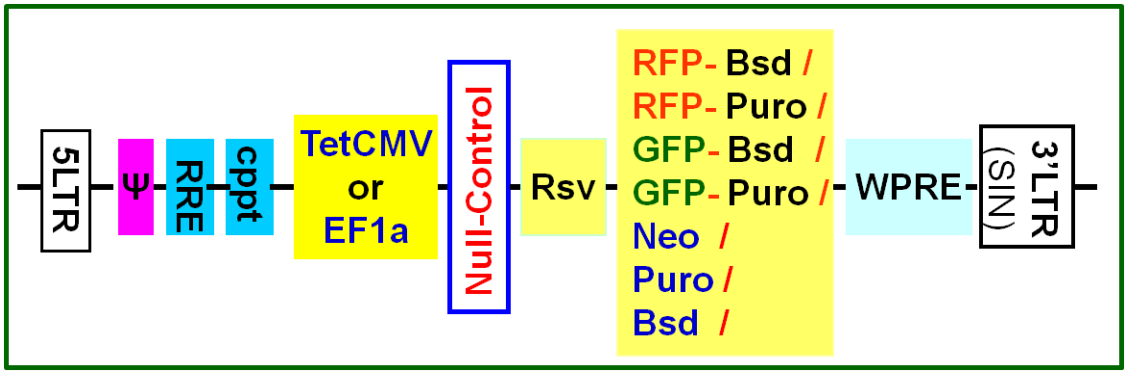
EF1a-Null-GB-PBS	EF1a Control lentiviral particles (GFP-Bsd) in PBS	
EF1a-Null-GP-PBS	EF1a Control lentiviral particles (GFP-Puro) in PBS	
EF1a-Null-RP-PBS	EF1a Control lentiviral particles (RFP-Puro) in PBS	
EF1a-Null-RB-PBS	EF1a Control lentiviral particles (RFP-Bsd) in PBS	

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

Product Description:

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.

GenTarget provides pre-made Lentiviral Particles expressing fluorescent proteins, enzymes, human or mouse target. The Lentiviral Particles are generated from an inducible lentivector under an enhanced CMV promoter, or from a constitutive, enhanced **EF1a promoter**. To serves as the negative controls for lentivirus treamtent, GenTarget also provides the Negative control lentivirus. The controls are made from the same lentivector backbone as the target expression vector, cloned with a Null spacer sequence (200bp) replacing the target sequence. The control lentivirus is packaged in the same way as any target expression virus, but control lentiviruses do not express a specific protein since the Null sequence does not contain a start codon. Please see the map below for each control vector structure.



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Control lentiviruses contain antibiotic markers matching that in our target expression lentiviruses. The control virus can be used alone to evaluate lentiviral transduction efficiency and for other applications.

VSV-G pseudotyped control lentiviruses are generated in 293T cells and provided as 200 µl per vial in either DMEM medium (containing 10% serum) or concentrated in PBS solution. For more details about premade particles, please see FAQs for pre-made lentiviral particles (.pdf).

Key features:

- High viral titer
- Different antibiotic selection
- Easy transduction monitoring by fluorescence
- Ready to use: simply add 50 µl per well in a 24-well plate.

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.

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, CO_2 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.

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Day 3:

At ~72hr 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, and select for antibiotic resistance. 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 necessary.

Measure cell density. When density has reached ~3 x 10^6 cells/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 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:
GFP filter: ~Ex450-490 ~Em525;
RFP filter: ~Ex545 ~Em620;

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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. Ware glove all the time at handling Lentiviral particles! Please refer CDC and NIH's guidelines for more details regarding to safety issues.

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.

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. NIH Guidelines for Biosafety Considerations for Research with Lentiviral Vectors. (Link).
9. CDC guidelines for Lab Biosafety levels (Link).

Related Products: GenTarget's Pre-made lentivirus Products:

Product Category	Product Description (please click category name to see product's pages)
Human, mouse or rat ORFs	Premade lentivirus expressin a **human, mouse or rat** gene with RFP-Blastididin fusion dual markers.
Fluorescent markers	Preamde lentivirus express human codon optimized fluorescent protein, **GFP / RFP/ CFP/ BFP / YFP**.
Luciferase expression	Premade lentivirus for all kinds of luciferase protein expression**: firefly and Renilla** with different antibiotic selection markers.
CRE recombinase	Premade lentivirus for expressing **nuclear permeant CRE** recombinase with different flurescent and antibiotic markers.

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LoxP ColorSwitch	Premade lentivirus expressing "LoxP-GFP-Stop-LoxP-RFP" cassette, used to monitor the CRE recombination event in vivo.
CRISPR /hu CAS9	Preamde lentivirus express humanzied wild-type **Cas9** endonuclease for genomic editing with **CRISPR**
TetR inducible expression repressor	Premade lentivirus expressin **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 antibitoic markers
T-antigen Expression	Express **SV40 large T antigen** with different selection markers
Cell Organelle imaging	Premade lentivirus for cell organelle imaging. The fluorescent marker **GFP/RFP/CFP was sub-cellular localized** in different cell organelle for living cell imaging.
LacZ expression	Express different full length **β- galactosidase (lacZ)** with different selection markers
Anti-miNA lentivirus	Pre-made lentivirus expression a specific **anti-miRNA** cassette.
Fluorescent-ORF fusion	Pre-made lentivirus expression a "**GFP/RFP/CFP-ORF**" fusion target.
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.
Other Enzyme expression	Ready-to-use lentivirus, expressing **specific enzymes** with different selection markers.