



## Pre-made Negative Control Lentiviral Expression Particles

Cat#	Product Name	Amounts
<a href="#">CMV-Null-Bsd</a>	CMV Control lentiviral particles (Bsd)	200ul/each, ~1 x 10 <sup>7</sup> IFU/mL in DMEM containing 10% FBS,
<a href="#">CMV-Null-Neo</a>	CMV Control lentiviral particles (Neo)	
<a href="#">CMV-Null-Puro</a>	CMV Control lentiviral particles (Puro)	
<a href="#">CMV-Null-GB</a>	CMV Control lentiviral particles (GFP-Bsd)	
<a href="#">CMV-Null-GP</a>	CMV Control lentiviral particles (GFP-Puro)	
<a href="#">CMV-Null-RP</a>	CMV Control lentiviral particles (RFP-Puro)	
<a href="#">CMV-Null-RB</a>	CMV Control lentiviral particles (RFP-Bsd)	
<a href="#">CMV-Null-Hygro</a>	CMV control lentivirus (Hygro)	
<a href="#">CMV-Null-Zeo</a>	CMV control lentivirus (Zeo)	
<a href="#">EF1a-Null-Bsd</a>	EF1a Control lentiviral particles (Bsd)	
<a href="#">EF1a-Null-Neo</a>	EF1a Control lentiviral particles (Neo)	
<a href="#">EF1a-Null-Puro</a>	EF1a Control lentiviral particles (Puro)	
<a href="#">EF1a-Null-GB</a>	EF1a Control lentiviral particles (GFP-Bsd)	
<a href="#">EF1a-Null-GP</a>	EF1a Control lentiviral particles (GFP-Puro)	
<a href="#">EF1a-Null-RP</a>	EF1a Control lentiviral particles (RFP-Puro)	
<a href="#">EF1a-Null-RB</a>	EF1a Control lentiviral particles (RFP-Bsd)	
<a href="#">EF1a-Null-Hygro</a>	EF1a control lentivirus (Hygro)	
<a href="#">EF1a-Null-Zeo</a>	EF1a control lentivirus (Zeo)	
<a href="#">CMV-Null-Bsd-PBS</a>	CMV Control lentiviral particles (Bsd) in PBS	200ul/each, ~1 x 10 <sup>8</sup> IFU/mL in PBS solution
<a href="#">CMV-Null-Neo-PBS</a>	CMV Control lentiviral particles (Neo) in PBS	
<a href="#">CMV-Null-Puro-PBS</a>	CMV Control lentiviral particles (Puro) in PBS	
<a href="#">CMV-Null-GB-PBS</a>	CMV Control lentiviral particles (GFP-Bsd) in PBS	
<a href="#">CMV-Null-GP-PBS</a>	CMV Control lentiviral particles (GFP-Puro) in PBS	
<a href="#">CMV-Null-RP-PBS</a>	CMV Control lentiviral particles (RFP-Puro) in PBS	
<a href="#">CMV-Null-RB-PBS</a>	CMV Control lentiviral particles (RFP-Bsd) in PBS	
<a href="#">EF1a-Null-Bsd-PBS</a>	EF1a Control lentiviral particles	



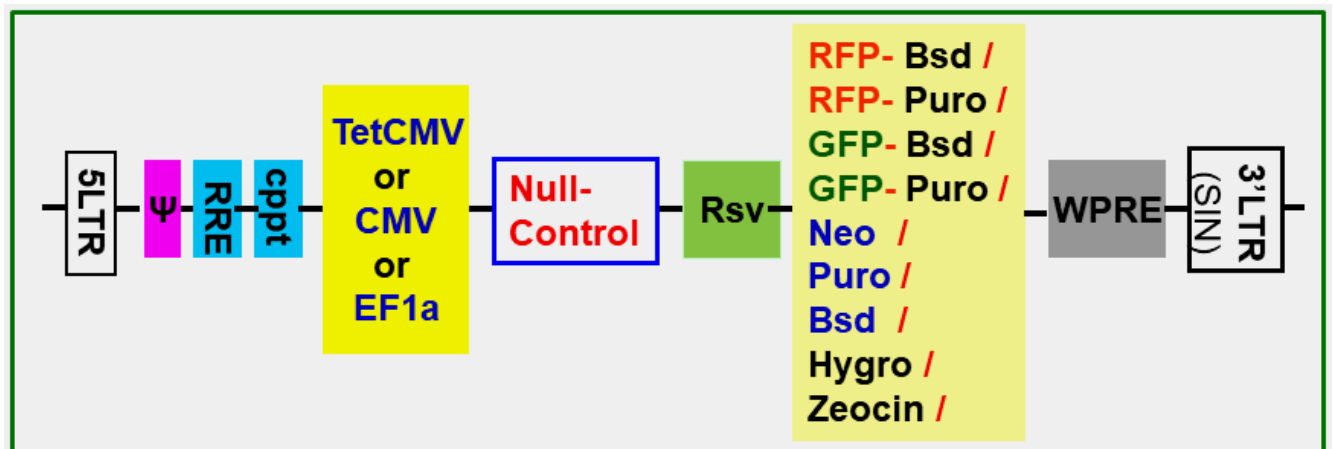
	(Bsd) in PBS	
<a href="#">EF1a-Null-Neo-PBS</a>	EF1a Control lentiviral particles (Neo) in PBS	
<a href="#">EF1a-Null-Puro-PBS</a>	EF1a Control lentiviral particles (Puro) in PBS	
<a href="#">EF1a-Null-GB-PBS</a>	EF1a Control lentiviral particles (GFP-Bsd) in PBS	
<a href="#">EF1a-Null-GP-PBS</a>	EF1a Control lentiviral particles (GFP-Puro) in PBS	
<a href="#">EF1a-Null-RP-PBS</a>	EF1a Control lentiviral particles (RFP-Puro) in PBS	
<a href="#">EF1a-Null-RB-PBS</a>	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 lentivirus expressing fluorescent proteins, enzymes, human or mouse target. The lentivirus are generated under an [inducible lentivector under an enhanced CMV promoter](#), or an enhanced constitutive CMV promoter, or an enhanced **EF1a promoter**. To serve as the negative controls for lentivirus treatment, 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 target because the Null sequence does not contain a start codon. Please see the map scheme above for the control lentivector core-structure.

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  $\mu$ l 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  $\mu$ l 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  $\mu$ 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  $0.5 \times 10^5$ /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<sub>2</sub> 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 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<sub>2</sub> incubator if necessary.

Measure cell density. When density has reached ~3 x 10<sup>6</sup> cells/ml, measured viability should be > 90%. Dilute cells into 1 x 10<sup>6</sup> 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 CO<sub>2</sub> 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 CO<sub>2</sub> 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;

**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 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:

<b>Lentivirus Category</b> (click to see)	<b>Product Description</b>
<a href="#">Target Expression</a>	Premade lentivirus express a <b>human, mouse or rat</b> gene with Fluorescent-Antibiotic fusion dual selection.
<a href="#">Luciferase expression</a>	Premade lentivirus express all kinds of luciferase: <b>firefly; Renilla; Cypridina; Red-Luc; Nano-Luc</b> , with different fluorescent and antibiotic selection.



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



<a href="#">iPS factors</a>	<b>NANOG, OCT4, SOX2, FGF4</b> ) factors with different fluorescent and antibiotic markers
<a href="#">LacZ expression</a>	Express different full length <b><math>\beta</math>-galactosidase (lacZ)</b> with different selection markers
<a href="#">Anti-miRNA lentivirus</a>	Pre-made lentivirus expression a specific <b>anti-miRNA</b> cassette.
<a href="#">Pre-made shRNA lentivirus</a>	Premade shRNA lentivirus for knockdown a specific genes ( <b>P53, LacZ, Luciferase</b> and more).
<a href="#">microRNA and anti-microRNA lentivirus</a>	Premade lentivirus expression human or mouse <b>precursor miRNA</b> . And <b>anti-miRNA</b> lentivector and virus for human and mouse miRNA.
<a href="#">Negative control lentiviruses</a>	Premade <b>negative control lentivirus with different markers</b> : serves as the negative control of lentivirus treatment, for validation of the specificity of any lentivirus target expression effects.
<a href="#">Other Enzyme</a>	Ready-to-use lentivirus, expressing <b>specific enzymes</b> with different selection markers.