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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

Cyno CD8a/CD8b Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to transduce almost all types of mammalian cells, including primary and non-dividing cells. These viruses transduce cells with a P2A-linked cynomolgus monkey (*Macaca fascicularis*) CD8a (XP_065382910.1) and CD8b (XP_005575417.1) driven by an EF1A promoter, and a puromycin selection marker (Figure 1).

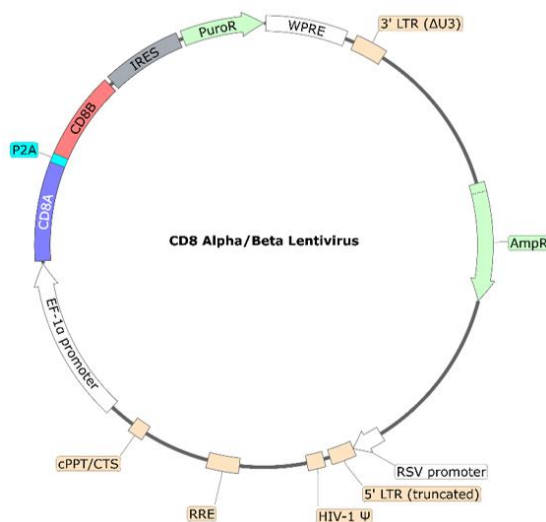


Figure 1. Schematic of the lenti-vector used to generate the Cyno CD8a/CD8b Lentivirus.

Background

CD8 (cluster of differentiation 8) is a cell surface glycoprotein found on most cytotoxic T lymphocytes that functions within the immune system to mediate cell-cell interactions. The functional forms of CD8 consist of either a heterodimer of two isoforms, CD8 α and CD8 β , or a homodimer of two CD8 α molecules. CD8 acts as a coreceptor to facilitate binding between the T cell Receptor (TCR) and the class I Major Histocompatibility Complex (MHC). Studying CD8 and its importance in isoimmunity can further our understanding of post-transplant recognition.

Application(s)

- Study CD8-MHC class I interaction.
- Generate Cyno CD8a and CD8b-expressing cell pools or stable cell lines following puromycin selection.

Formulation

The lentivirus particles were produced in HEK293T cells in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations by special request, for an additional fee.

Size and Titer

Two vials (500 μ l x 2) of lentivirus at a titer $\geq 10^7$ TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage



Lentiviruses are shipped with dry ice. For long-term storage, it is recommended to store the lentiviruses at -80°C for up to 12 months from date of receipt. Avoid repeated freeze/thaw cycles. Titers can drop significantly with each freeze/thaw cycle.

Biosafety

The lentiviruses are produced with a SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and after integration into the genomic DNA of the target cells. None of the HIV genes (*gag*, *pol*, *rev*) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal and are not present in the lentivirus particle. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS Bioscience recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied

These materials are not supplied with this lentivirus but are necessary to follow the protocol described in the “Validation Data” section. Media and reagents used at BPS Bioscience are all validated and optimized for use with this lentivirus and are highly recommended for best results.

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
6-well tissue culture-treated assay plates	
Flow cytometer	

Assay Protocol

The following Protocol is a general method for transducing Jurkat cells. The optimal transduction conditions (e.g., MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the target gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the target with the appropriate antibiotic.

Day 1:

1. Harvest Jurkat cells by centrifugation and resuspend the cells in fresh Thaw Medium 2.
2. Dilute the cells to 5×10^5 /ml in Thaw Medium 2.
3. Mix 750 μ l of the Jurkat cells and 250 μ l of Cyno CD8a/CD8b Lentivirus in a 1.5-ml Eppendorf tube.
4. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to a final concentration of 8 μ g/ml.
5. Gently mix and incubate the virus with the Jurkat cells for 20 min at room temperature in the tissue culture hood.
6. Centrifuge the virus/cells mixture for 30 minutes at $800 \times g$ at 32°C.
7. Remove the virus-containing medium and resuspend the cell pellet in 3 ml of fresh Thaw Medium 2.
8. Transfer the cells into one well in a 6-well plate.
9. Incubate the plate at 37°C with 5% CO₂ for 48-72 hours.

Day 3-4:

1. The transduced Jurkat cells are ready for flow cytometry analysis or another method of interest.

Important Notes

To generate a Cyno CD8a-CD8b stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin (as pre-determined from a killing curve, <https://bpsbioscience.com/kill-curve-protocol>), for antibiotic selection of transduced cells followed by clonal selection.

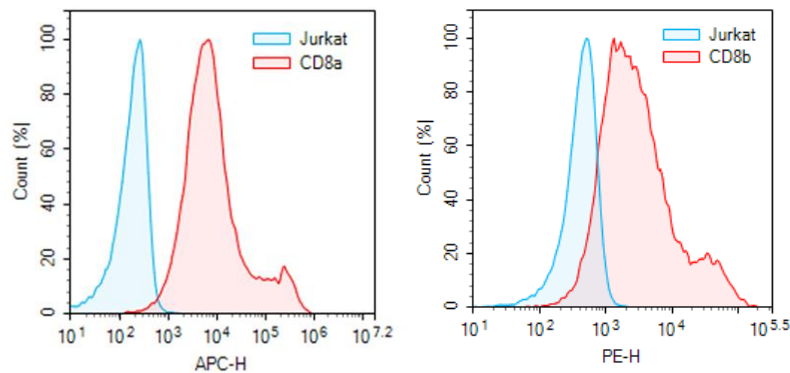
Figures and Validation Data

Figure 2. Expression of Cyno CD8a and CD8b in Jurkat cells transduced with Cyno CD8a/CD8b Lentivirus by flow cytometry.

Approximately 100,000 cells/well of Jurkat cells were transduced with 1,000,000 TU/well of CD8a/CD8b Lentivirus using spinoculation. 48 hours post-transduction, the expression of CD8a and CD8b in the target cells was analyzed by flow cytometry using APC- anti-human CD8 Antibody (Biolegend #344722; left panel) and PE anti-human CD8b Antibody (Biolegend #376703; right panel). The y axis indicates the % of cells and the x axis the fluorophore intensity.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

Visit bpsbioscience.com/lentivirus-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
CD8a Lentivirus	78648	500 µl x 2
CD8a/CD8b Lentivirus	78650	500 µl x 2

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