SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

Description

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, CD8α and CD8β, or a homodimer of two CD8α 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.

The CD8a/CD8b (CD8α/CD8β) Lentiviruses 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. The particles contain P2A-linked CD8a (NM_001768.6) and CD8b (NM_004931.5) driven by an EF1a promoter and a puromycin selection marker (Figure 1).

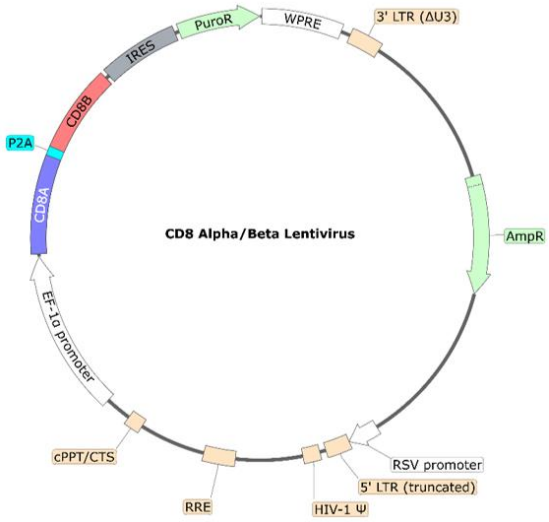

Figure 1. Schematic of the lenti-vector used to generate the CD8a/CD8b Lentivirus.

Application(s)

- Study CD8-MHC class I interaction.
- Generation of a stable cell line expressing CD8a and CD8b with puromycin selection.

Formulation

The lentivirus particles were produced from HEK293T cells. They are supplied in cell culture medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of lentivirus at a titer ≥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. Avoid repeated freeze/thaw cycles. Titers can drop significantly with each freeze/thaw cycle.

BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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.

Assay Protocol

The following **Protocol 1** is a general method for transducing adherent cell lines, such as HEK293, CHO, or Hela. The following **Protocol 2** (Spinoculation) is recommended for transducing suspension cells, such as Jurkat, THP-1, PMBC etc.

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 gene with the appropriate antibiotic.

Protocol 1:

This protocol is a general method for transducing adherent cell lines, such as HEK293, CHO, or Hela.

1. Day 1: Seed HEK293 cells at a density of 100,000 cells per well into 12-well microplate in 1000 µl of Thaw Medium 1 (BPS Bioscience #60187). Add 10 µl of CD8a/CD8b lentivirus into each well. Optional: Add polybrene to each well at a final concentration of 5 µg/ml.

   Gently swirl the plate to mix. Incubate the plate at 37°C with 5% CO_2 overnight.

2. Day 2: Remove the medium containing the lentivirus from the wells. Add 1000 µl of fresh Thaw Medium 1 to each well.

   If neither the polybrene nor the lentivirus adversely affects the target cells, it is not necessary to change the medium on Day 2. The target cells can be incubated with the virus for 48-72 hours before changing the medium.

3. Day 3-4, approximately 48-72 hours after transduction, the expression of CD8a and CD8b in the target cells can be examined by flow cytometry.

Protocol 2:

This protocol (Spinoculation) is recommended for transducing suspension cells, such as Jurkat, THP-1, PMBC etc.

1. Harvest Jurkat cells by centrifugation and resuspend the cells in fresh Thaw Medium 2 (BPS Bioscience #60184). Dilute the cells to 5 x 10^5 /ml in growth medium. Mix 750 µl of the Jurkat cells and 250 µl of lentivirus in a 1.5-ml Eppendorf tube. Add polybrene to a final concentration of 8 µg/ml. Gently mix and incubate the virus with the Jurkat cells for 20 min at room temperature in the tissue culture hood.

2. Centrifuge the virus/cells mixture for 30 minutes at 800 x g at 32°C. Remove the virus containing medium and resuspend the cell pellet in 3 ml of fresh Thaw Medium 2. Transfer the cells into one well in a 6-well plate. Incubate the plate at 37°C with 5% CO_2 for 48-72 hours.

3. The transduced Jurkat cells are ready for flow cytometry analysis.

Notes

To generate a 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) for antibiotics selection of transduced cells. Visit: https://bpsbioscience.com/cell-line-faq for guidelines on performing a kill curve.

Figures and Validation Data

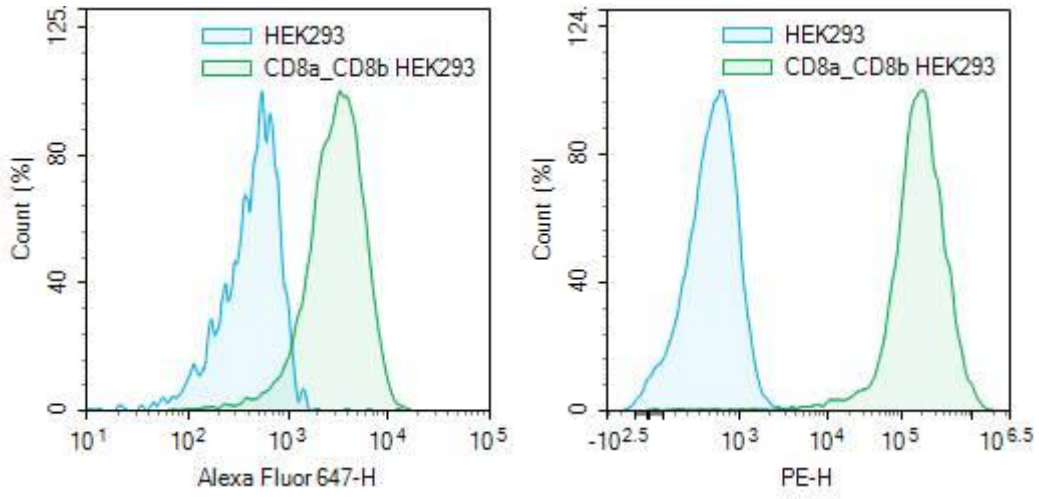

Figure 2. Transduction of HEK293 cells using CD8a/CD8b lentivirus.
Approximately 100,000 cells/well of HEK293 cells were transduced with 200,000 TU/well of CD8a/CD8b lentivirus. After 66 hours of transduction, the expression of CD8a and CD8b in the target cells was analyzed by flow cytometry using Alexa Fluor® 647 anti-human CD8 Antibody (Biolegend #344725; left panel) and PE anti-human CD8b Recombinant Antibody (Biolegend #376703; right panel).

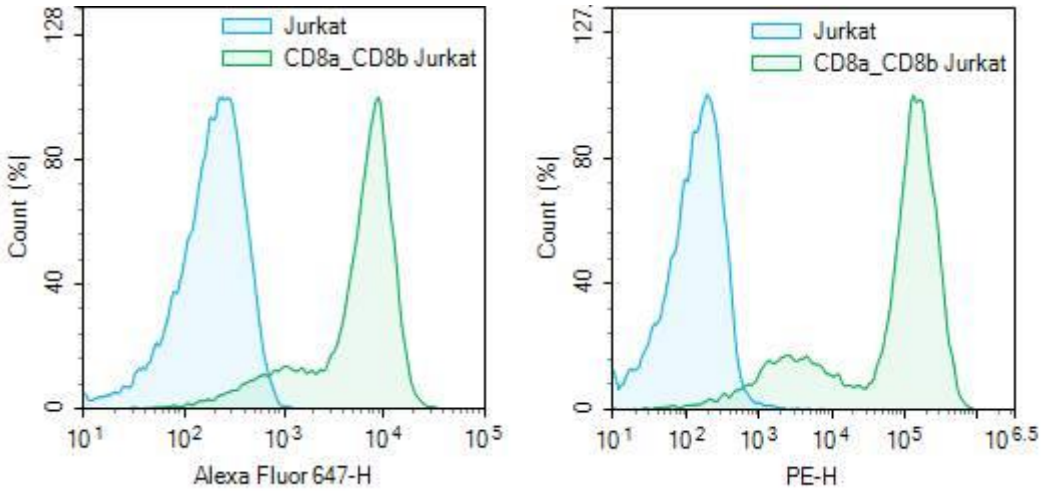

Figure 3. Transduction of Jurkat cells using CD8a/CD8b lentivirus.
Approximately 100,000 cells/well of Jurkat cells were transduced with 1,000,000 TU/well of CD8a/CD8b lentivirus using spinoculation. After 48 hours of transduction, the cells were grown in growth medium containing 0.5 µg/mL puromycin for one week. The expression of CD8a and CD8b in the target cells was analyzed by flow cytometry using Alexa Fluor® 647 anti-human CD8 Antibody (Biolegend #344725; left panel) and PE anti-human CD8b Recombinant Antibody (Biolegend #376703; right panel).

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

Related Products

Products	*Catalog #*	*Size*
eGFP Lentivirus (Inducible TET On)	78629	500 µl x 2
Enhanced GFP Lentivirus (G418)	78639-G	500 µl x 2
Enhanced GFP Lentivirus (Hygro)	78639-H	500 µl x 2
Firefly Luciferase Lentivirus	79692	500 µl x 2
Renilla Luciferase Lentivirus	79565	500 µl x 2
Secreted Gaussia Lentivirus	79892	500 µl x 2
Non-Secreted Gaussia Lentivirus	79893	500 µl x 2
YFP Lentivirus	79989	500 µl x 2
RFP Lentivirus	78347	500 µl x 2
CD8a Lentivirus	78648	500 µl x 2