Description

The Human CD4 Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain CD4 (NM_000616.5) driven by an EF1a promoter, and a hygromycin selection marker (Figure 1).

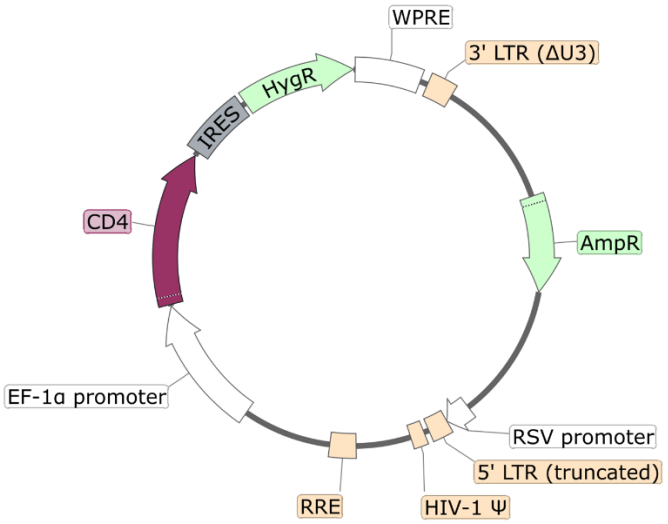

Figure 1. Schematic of the lenti-vector used to generate the Human CD4 Lentivirus.

Background

CD4 is a cell surface glycoprotein found on partially defined functional T-cell subsets, including helper T cells and T-regulatory cells, peripheral monocytes and other APCs (antigen-presenting cells). The ectodomain of CD4 binds to membrane-proximal domains of MHC (major histocompatibility complex) class II molecules, while its cytoplasmic domains interact with the protein tyrosine kinase p56lck (lck) through a shared cysteine-containing motif. $CD4^+$ T cells, or helper T cells, are one type of lymphocyte that helps coordinate the immune response against infection and disease by activating cells of the innate immune system, B-lymphocytes and cytotoxic T cells. $CD4^+$ T cells are activated by interaction between the TCR (T- cell receptor) and its cognate peptide presented on MHC II molecules, and CD4 is a critical component of the T cell receptor complex that recognizes antigenic peptides presented by MHC II molecules, increasing its stability. CD4 is a typically used T cell marker that allows to characterize the populations of T cells present.

Application

- Study CD4-MHC class II interactions.
- Generation of cell pools or stable cell lines expressing CD4 following hygromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells. Supplied in medium containing 90% DMEM + 10% FBS. Virus particles can be packaged in custom formulations and produced at higher titers by special request, for an additional fee.

Size and Titer

Two vials (500 µl x 2) of lentiviruses 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
Cells of interest	
Thaw Medium 2	BPS Bioscience #60184
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
96-well tissue culture-treated assay plates	
Flow cytometer	

Assay Protocol

- The following protocol, a spinoculation protocol, is recommended for transducing cells that grow in suspension, such as Jurkat and THP-1 cells, and PBMC (peripheral blood mononuclear cells).
- The following protocol is a general guideline 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 hygromycin prior to carrying out the assays.

Day 1:

1. Harvest Jurkat cells by centrifugation and resuspend the cells in fresh Thaw Medium 2.
2. Count cells and dilute the cell suspension to 5 x 10^5 cells/ml in Thaw Medium 2.
3. Mix 750 µl of the Jurkat cell suspension with 250 µl of Human CD4 Lentivirus in a 1.5-ml Eppendorf tube.
4. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to a final concentration of 8 µg/ml.

5. Gently mix and incubate the viruses with the Jurkat cells for 20 minutes at Room Temperature (RT) in a tissue culture hood.

6. Centrifuge the viruses/cells mixture for 30 minutes at 800 *x g* and 32°C.

7. Remove the virus containing medium and resuspend the cell pellet obtained in 3 ml of fresh Thaw Medium 2.

8. Transfer the cells into one well in a 6-well plate and incubate the plate at 37°C with 5% CO_2 for 48-72 hours.

Day 3-4:

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

Notes

To generate a CD4 expressing stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of hygromycin (as pre-determined from a killing curve, https://bpsbioscience.com/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

Figures and Validation Data

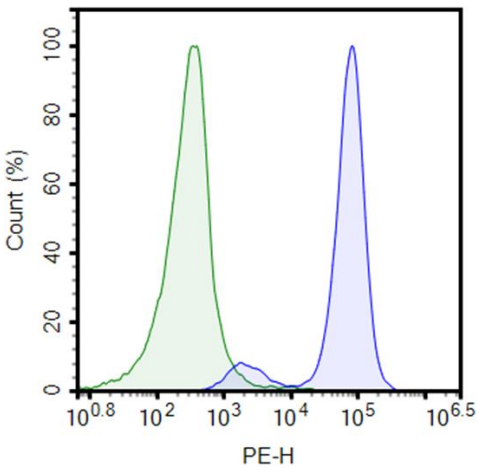

Figure 2. CD4 expression in Jurkat cells transduced with Human CD4 Lentivirus analyzed by flow cytometry.
Approximately 100,000 Jurkat cells/well were transduced with 1,000,000 TU/well of Human CD4 Lentivirus particles by spinoculation. 48 hours post- transduction, the cells were grown in growth medium containing 100 µg/ml hygromycin for one week, and the antibiotic-resistant cell pool was stained with Anti-CD4, PE-Labeled (#102010) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity.

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

Sequence

Human CD4 sequence (NM_000616.5)

MNRGVPFRHLLLVLQLALLPAATQGKKVVLGKKGDTVELTCTASQKKSIQFHWKNSNQIKILGNQGSFLTKGPSKLNDRADSRRS
LWDQGNFPLIIKNLKIEDSDTYICEVEDQKEEVQLLVFGLTANSDTHLLQGQSLTLTLESPPGSSPSVQCRSPRGKNIQGGKTLSVS
QLELQDSGTWTCTVLQNQKKVEFKIDIVVLAFQKASSIVYKKEGEQVEFSFPLAFTVEKLTGSGELWWQAERASSSKSWITFDLKN
KEVSVKRVTQDPKLQMGKKLPLHLTLPQALPQYAGSGNLTLALEAKTGKLHQEVNLVVMRATQLQKNLTCEVWGPTSPKLMLSL
KLENKEAKVSKREKAVWVLNPEAGMWQCLLSDSGQVLLESNIKVLPTWSTPVQPMALIVLGGVAGLLLFIGLGIFFCVRCRHRRR
QAERMSQIKRLLSEKKTCQCPHRFQKTCSPI

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*
CD8a Lentivirus	78648	500 µl x 2
CD8a/CD8b Lentivirus	78650	500 µl x 2
Anti-CD4, PE-Labeled	102010	25 µg/100 µg
Anti-CD8, PE-Labeled	102011	25 µg/100 µg
$CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78757	2 vials

Version 051724