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Data Sheet

TCR Activator Lentivirus (EF1a Promoter/Puromycin)

Catalog #: 79894-EP

Product Description

The TCR Activator 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 a gene for a membrane-bound, engineered T cell receptor (TCR) activator driven by an EF1A promoter (Figure 1). After transduction, the expression of TCR activator can be monitored in the target cells.

Application

1. Expression of TCR activator in target cells to stimulate T cells
2. Generation of a stable cell line expressing TCR activator with puromycin selection.

Formulation

The lentiviruses were produced from HEK293T cells in medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of TCR Activator Lentivirus at a titer ≥10^7 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

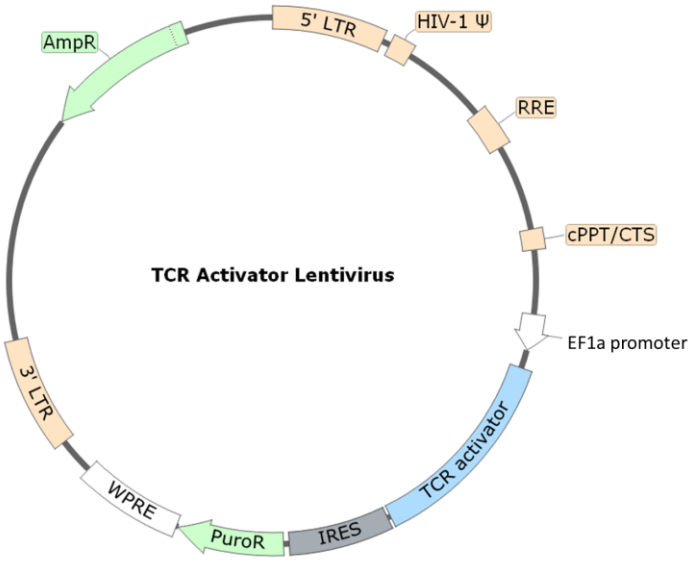

Figure 1. Schematic of the lenti-vector used to generate the TCR Activator Lentivirus

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Storage
Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

Biosafety
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. Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local, federal, state, and institutional regulations and using all appropriate safety precautions.

Materials Required but Not Supplied
- CHO-K1 cells (ATCC, #CCL-61)
- CHO growth medium or use BPS Thaw Medium 3 (BPS Bioscience, #60186)
- NFAT reporter (Luc) Jurkat cell line (BPS Bioscience, #60621)
- Jurkat growth medium or use BPS Thaw Medium 2 (BPS Bioscience, #60184)
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

The following protocol is a general guideline for transducing CHO-K1 cells using TCR Activator Lentivirus. 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 TCR activator can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing TCR activator with puromycin prior to carrying out the assays.

1. Day 1: Harvest CHO-K1 cells from culture and seed cells at a density of 5,000-10,000 cells per well into white opaque 96-well microplate in 50 µl of CHO growth medium. Incubate cells at 37°C with 5% CO_2 overnight.

   Meanwhile, maintain NFAT reporter (Luc) Jurkat cells according to the manufacturer's protocol for TCR activator assay.

2. Day 2: To each well add 5 µl of TCR Activator Lentivirus or Expression Negative Control Lentivirus (BPS Bioscience, #79902-P). 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 for 18-24 hours.
   Alternatively, seeding cells and the transduction can be performed on the same day.

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3. Day 3: Remove the medium containing the lentivirus from the wells. Add 100 µl of fresh CHO growth medium 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 3. The target cells can be incubated with the virus for 48-72 hours before changing medium.

4. On the morning of Day 5, harvest NFAT reporter (Luc) Jurkat cells (log phase) by centrifugation and resuspend the cells in Jurkat growth medium. Dilute the cells to $3x10^5$ in Jurkat growth medium. Remove the medium from TCR activator lentivirus transduced CHO cells, and add 100 µl of diluted NFAT reporter (Luc) Jurkat cells into each well.

   Incubate the plates at 37°C in a CO_2 incubator for 5 to 6 hours.

5. After ~5 to 6-hour incubation, perform luciferase assay using the ONE-Step luciferase assay system: Prepare ONE-Step Luciferase reagent as directed add 100 µl per well. aRock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer. If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

Important Notes:

To generate a TCR activator stable cell line, on day 4 remove CHO growth medium and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

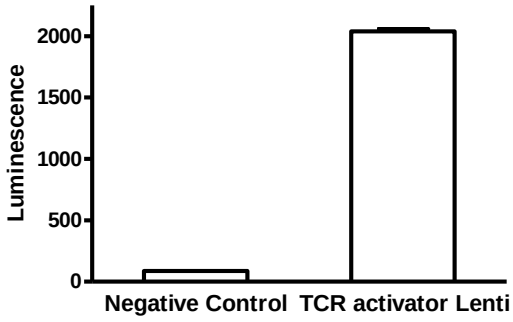

Figure 2. TCR activator activity in CHO-K1 cells transduced with TCR Activator Lentivirus. 10,000 CHO cells/well were transduced with 50,000 TU/well TCR Activator Lentivirus or Expression Negative Control Lentivirus in CHO growth medium. After 18 hours of transduction, medium was changed to fresh CHO growth medium. After 66 hours of transduction, transduced CHO cells were cocultured with NFAT reporter (Luc) Jurkat cells (BPS Bioscience, #60621) for 6 hours. The luciferase assay was performed using the ONE-Step™

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Luciferase assay system (BPS Bioscience, #60690), following the recommended protocol in the user manual.

Related Products

Product	Cat. #	Size
NFκB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
ISRE Luciferase Reporter Lentivirus	79824	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x2
AP-1 Luciferase Reporter Lentivirus	79823	500 µl x2
SBE Luciferase Reporter Lentivirus	79806	500 µl x2
TEAD Luciferase Reporter Lentivirus	79833	500 µl x2
ARE Luciferase Reporter Lentivirus	79869	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
FcGRIIIA Lentivirus	79876	500 µl x2
FcGRIIB Lentivirus	79877	500 µl x2
FcER1G Lentivirus	79878	500 µl x2
Expression Negative Control Lentivirus	79902	500 µl x2
Secreted Gaussia Lentivirus	79892	500 µl x2
Non-Secreted Gaussia Luciferase Lentivirus	79893	500 µl x2