Description

The Influenza A Virus HA TCR Lentivirus (Clone HA1.7) 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 particles transduce cells with human TCR (T-cell receptor) clone HA1.7 that specifically recognizes the Influenza A virus (or IAV) HA (hemagglutinin) peptide (PKYVKQNTLKLAT) in the context of MHC (major histocompatibility complex) class II molecules. The Influenza A Virus HA TCR α chain and β chain are linked by P2A. The lentiviruses also transduce a puromycin selection marker (Figure 1).

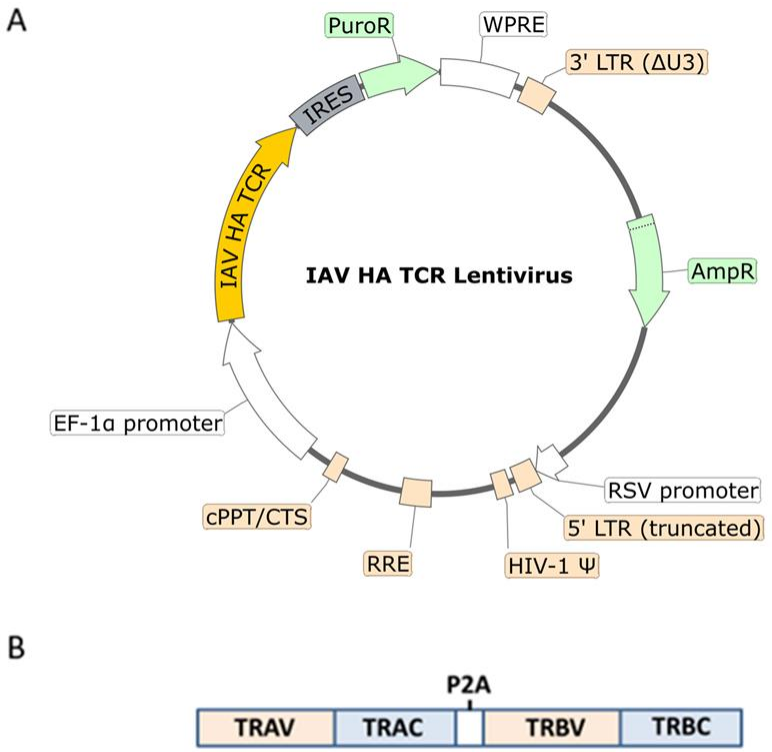

Figure 1. (A) Schematic of the lenti-vector used to generate the Influenza A Virus HA TCR Lentivirus (HA1.7 Clone). (B) Construct diagram showing expressed components of the IAV HA-specific TCR. TRAV and TRAC correspond to the TCR alpha chain variable and constant regions, respectively, whereas TRBV and TRBC correspond to the TCR beta chain variable and constant regions.

Background

Influenza A virus (IAV) is a virus that causes the flu in birds and mammals, including humans. Influenza A viruses are negative-sense, single-stranded, segmented RNA viruses whose subtypes are labeled according to an H number (for the type of hemagglutinin) and an N number (for the type of neuraminidase). There are 18 different known H antigens (H1 to H18) and 11 different known N antigens (N1 to N11), classified based on their reaction to antisera. Hemagglutinin binds to sialic acid on the surface of the target cells, and it is thus critical for infection. Neuraminidase is an enzyme that cleaves sialic acid and allows newly formed viruses to be released from the infected cells. The alpha/beta T cell receptor (TCR) HA1.7 specifically recognizes the influenza A virus hemagglutinin (HA) peptide (PKYVKQNTLKLAT) presented by the allo-major histocompatibility complex (MHC) class II molecule. Influenza is responsible for more than 36, 000 deaths/year in the USA only, and costs about 10 billion of dollars. It is thus crucial to continue to investigate and develop tools to minimize the impact of this virus on human health.

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Application

- Use as a positive control for IAV HA TCR evaluation and optimize experimental conditions for influenza research.
- Generate IAV HA TCR expression cell pools or stable cell lines, following puromycin selection.

Formulation

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

Titer

≥2 x 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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Name	Ordering Information
Thaw Medium 6	BPS Bioscience #60183
Growth Medium 2C	BPS Bioscience #79592
Assay Medium 2D	BPS Bioscience #78755
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78556
$CD4^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	BPS Bioscience #82319
A375 Cells	ATCC #CRL-1619
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
Influenza HA H3 Peptide (307-319)	BPS Bioscience #82311
Influenza HA H1 Peptide (Hawaii H1N1)	BPS Bioscience #82312
PE anti-human α/β T Cell Receptor Antibody	BioLegend #306707
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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Media Formulations

For best results, the use of BPS Bioscience validated and optimized media *is highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for Maintaining CD4$^+$ TCR Knockout NFAT-Luciferase Reporter Cell Line

Growth Medium 2C (BPS Bioscience #79592):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin, 100 µg/ml Hygromycin B.

Media Required for Maintaining A375 Cell Line

Thaw Medium 6 (BPS Bioscience #60183):
DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Media Required for Co-culture Assay

Assay Medium 2D (BPS Bioscience #78755):
RPMI 1640 medium supplemented with 1% FBS.

Assay Protocol

- The following protocol was used to transduce a Jurkat cell line. The 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 reporter 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 reporter gene with puromycin prior to carrying out the reporter assays.
- The assay should include “Peptide Loaded” and “Unloaded Control” wells.

Day 1:

1. Harvest CD4$^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells from Growth Medium 2C by centrifugation, resuspend the cells in fresh Thaw Medium 2 and count.

2. Dilute cells to a density of 2 x 10^5 cells/ml in Thaw Medium 2.

3. Mix 1 ml of the Jurkat cell suspension and appropriate amount of Influenza A Virus HA TCR Lentivirus (Clone HA1.7) in a 1.5-ml Eppendorf tube to obtain an MOI > 10.

4. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to a final concentration of 8 µg/ml.

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

6. Centrifuge the virus/cell mixture for two hours at 800 *x g* and 32°C (spinoculation).

7. Add the cells/virus mix from the spinoculation step to one well of a 6-well plate.

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8. Add an additional 1.5 ml of Thaw Medium 2 to the well.

   Note: It is not necessary to remove the virus.

9. Incubate the cells at 37°C with 5% CO_2 for 48-66 hours.

Day 3-4:

1. The expression of TCR can be analyzed by flow cytometry. The transduced Jurkat cells are ready for assay development on day 3 or 4.

   Note: If the transduction efficiency is low, it may be necessary to perform cell selection with puromycin on day 3.

2. Plate A375 cells as APCs (Antigen Presenting Cells) at a density of 2 x 10^4 cells/well to test the TCR-mediated reporter activation.

3. Incubate overnight at 37°C with 5% CO_2.

Day 4-5:

1. For use in the following co-culture assay at day 4-5 prepare materials and conditions as follows:

   a) Preparation of Antigenic-Mimetic Peptides:
      Thaw the peptide at RT.
      Dilute the peptide with Assay Medium 2D so that it is 2-fold higher than the desired final concentration (50 µl/ well).

      Note: The peptide stock was dissolved in DMSO at a concentration of 1 mM. The final DMSO concentration in the co-culture assay should not be >0.3%.

   b) Preparation of Antigen Presenting Cells (APCs):
      Remove the media from plated A375 cells.
      Add 50 µl of diluted peptide to the "Peptide Loaded" wells.
      Add 50 µl of Assay Medium 2D to the "Unloaded Control" wells (for measuring the basal luciferase activity).

2. Resuspend TCR-transduced $CD4^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells into Assay Medium 2D at a density of 5 x 10^5 cells/ml (50 µl/well).

3. Add 50 µl of TCR-transduced $CD4^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells into each well of the 96-well plate containing the APCs.

4. Incubate the co-culture plate at 37°C with 5% CO_2 for 5-6 hours or overnight.

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Day 5-6:

1. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

2. Incubate at RT for ~15 to 30 minutes.

3. Measure luminescence using a luminometer.

Notes

To generate a IAV HA TCR expressing 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/cell-line-faq), for antibiotic selection of transduced cells, followed by clonal selection.

Validation Data

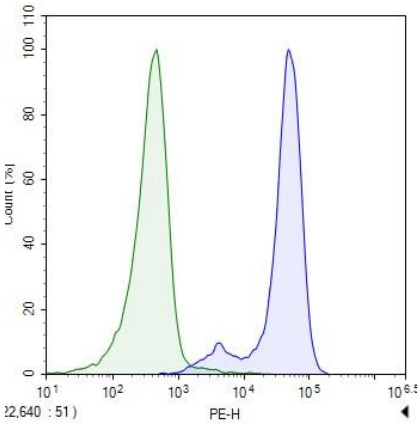

Figure 2. Expression of IAV HA TCR in Jurkat cells transduced with Influenza A Virus HA TCR Lentivirus (Clone HA1.7).

Approximately 100,000 $CD4^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #82319) were transduced with Influenza A Virus HA TCR Lentivirus (Clone HA1.7) (BPS Bioscience #78988) by spinoculation at a MOI of 10. 48 hours post-transduction, the cells were transferred into a medium containing 0.5 µg/ml of puromycin. After one week of antibiotic selection, the cells pool was stained with PE anti-human T Cell Receptor Antibody (Biolegend #306707) and the expression of IAV HA TCR was analyzed by flow cytometry (blue). Non-transduced cells were used as control (green). The y axis represents the % of cells. The x axis indicates fluorophore intensity.

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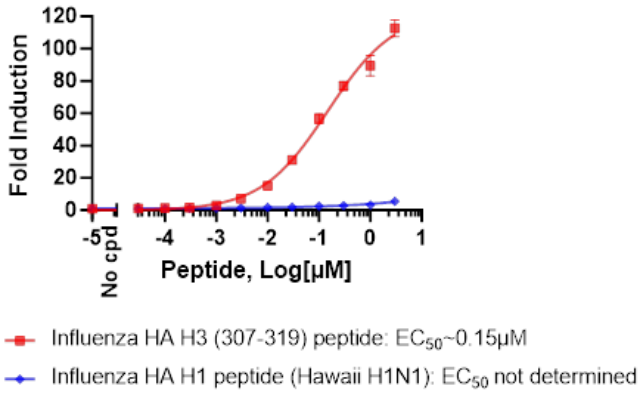

Figure 3. Jurkat T Cell activation after transduction with Influenza A Virus HA TCR Lentivirus (Clone HA1.7), using A375 cells as Antigen presenting cells (APCs).
$CD4^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #82319) were transduced with Influenza A Virus HA TCR Lentivirus (Clone HA1.7) (BPS Bioscience #78988) by spinoculation at a MOI of 10. 48 hours post-transduction, the cells were transferred into a medium containing 0.5 µg/ml of puromycin. After one week of antibiotic selection, the cell pool was co-cultured with A375 cells (ATCC #CRL-1619) loaded with Influenza HA H3 Peptide (307-319) (BPS Bioscience #82311) or control Influenza HA H1 Peptide (Hawaii H1N1) (BPS Bioscience #82312) for 6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System and correlates with T Cell Activation.

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

References

Hennecke J. and Wiley D.C., 2002 *J Exp Med.* 195:571–581.
Hennecke J., *et al.*, 2000 *EMBO J.* 19:5611–5624.
Lamb J.R., *et al.*, 1982 *Nature.* 300:66–69.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-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

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