Description

KRAS G12D-Specific TCR Lentivirus (Clone 10) are replication incompetent, HIV-based, VSV-G-pseudotyped lentiviral particles ready to transduce nearly all types of mammalian cells, including primary and non-dividing cells. These viruses transduce cells with a TCR (T cell receptor) (clone 10) that specifically recognizes the human antigen KRAS (Kirsten rat sarcoma virus) G12D, and in which the TCR α chain and β chain are linked by P2A. The lentiviruses also transduce a puromycin selection marker (Figure 1). Mouse TCR constant regions were used in this construct to boost expression.

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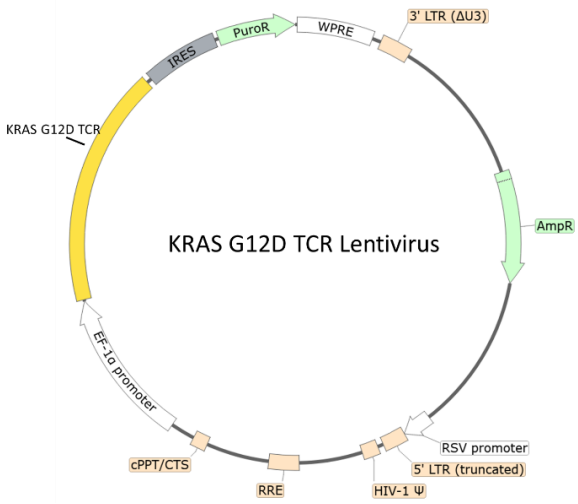

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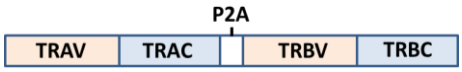

Figure 1. (A) *Schematic of the lenti-vector used to generate the KRAS G12D-Specific TCR Lentivirus (Clone 10) and (B) diagram of the construct, showing the components of the KRAS G12D-specific TCR.* TRAV and TRAC correspond to the TCR alpha chain variable and constant regions, respectively, whereas TRBV and TRBC correspond to the TCR beta chain variable and constant regions. Mouse TCR constant regions were used in this construct to boost expression.

Background

KRAS (Kirsten rat sarcoma virus) are GTPase proteins. They cycle between a GDP-bound inactive state and a GTP-bound (active) form, in a process regulated by two accessory proteins: GEF (guanine exchange factors) and GAPs (GTPase activating proteins). Once activated KRAS can bind to its effectors and regulate multiple signaling pathways, such as the RAF (rapidly accelerated fibrosarcoma)-MEK (mitogen activated protein kinase)-ERK (extracellular regulated kinase) or the PI3K (phosphoinositide 3-kinase)-AKT (protein kinase B)-mTOR (mammalian target of rapamycin) signaling pathways. KRAS mutations account for about 85% of all RAS mutations and are considered one of the main drivers of human cancer, such as in PDAC (pancreatic ductal adenocarcinoma). One of the amino acids frequently mutated is glycine 12, with the most common form being G12D. Since KRAS are intracellular proteins, they are not amenable to CAR (chimeric antigen receptor)-T cell-based therapies, and the development of inhibitors has also proved challenging. One strategy involves the use of TCR (T cell receptor)-T cells, targeting this antigen. Specific TCR clones have been identified, with a KRAS G12D-specific TCR (clone 9c) preferentially being reactive against KRAS G12D peptide (10-18, 9mer), in comparison with KRAS G12D peptide (10-19, 10mer) and being unable to recognize the wild-type KRAS peptides. On the other hand, a KRAS G12D-specific TCR (clone 10) is preferentially reactive against KRAS G12D peptide (10-19, 10mer), in comparison with KRAS G12D peptide (10-18, 9mer) and it does not recognize wild-type KRAS peptides. Results from a trial using a KRAS G12D HLA-C*08:02 restricted TCR demonstrated the potential of this approach for the treatment of PDAC. The use of neoantigen specific TCR-T therapies, targeting single amino acid mutations, is thus an exciting and promising cancer therapy.

Application

- Use as a positive control for KRAS G12D TCR evaluation and optimize experimental conditions.
- Generate KRAS G12D TCR expressing cell pools or stable cell lines, following puromycin selection.

Formulation

The lentiviruses 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.

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 virus 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 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. 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 designed protocol. BPS Bioscience media, reagents, and luciferase assay systems 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
Growth Medium 2C	BPS Bioscience #79592
Assay Medium 2D	BPS Bioscience #78755
CD8$^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78757
HLA-C*08:02 K562 Cell line	BPS Bioscience #78974
KRAS G12D Peptide (10-18, 9mer)	BPS Bioscience #78967
KRAS Wild Type Peptide (10-18, 9mer)	BPS Bioscience #78968
KRAS G12D Peptide (10-19, 10mer)	BPS Bioscience #78969
KRAS Wild Type Peptide (10-19, 10mer)	BPS Bioscience #78970
PE anti-mouse TCR β chain Antibody	BioLegend #109207
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939

Media Formulations

For the 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 CD8$^+$ 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 HLA-C*08:02 K562 Cell Line*

Thaw Medium 2E (BPS Bioscience #79638):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 µg/ml Puromycin.

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 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, with puromycin, prior to carrying out the reporter assays.
- The assay should include “Peptide Loaded” and “Unloaded Control” wells.

Day 1:

1. Harvest CD8$^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells from Growth Medium 2C by centrifugation and resuspend the cells in fresh Thaw Medium 2.

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

3. Mix 1 ml of Jurkat cell suspension with the appropriate amount of KRAS G12D-Specific TCR Lentivirus (Clone 10) in a 1.5-ml Eppendorf tube to obtain an MOI>10.

4. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancerto 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 30-120 minutes at 800 *x g* at 32°C (spinoculation).

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

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

   Note: It is not necessary to remove the viruses.

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 initiate cell selection with puromycin on day 3.

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

   a) Preparation of Antigenic-Mimetic Peptides:
   Thaw the KRAS G12D peptide at RT.
   Dilute the peptide with Assay Medium 2D so that it is 5-fold higher than the desired final concentration.

   Note: The peptide stock was dissolved in DMSO to 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):
   Harvest HLA-C*08:02 K562 Cell line from Thaw Medium 2E and resuspend the cells into Assay Medium 2D at a density of 5 x 10^5/ml.
   Add 40 µl of HLA-C*08:02 K562 cells into each well of a 96-well plate.
   Add 20 µl of diluted peptide to the “Peptide Loaded” wells.
   Add 20 µl of Assay Medium 2D to the “Unloaded Control” wells (for measuring the basal luciferase activity).

   c) Resuspend Jurkat cells into Assay Medium 2D at a density of 4 x 10^5/ml.
   Add 40 µl of TCR-transduced $CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells into each well of the 96-well plate containing the APCs.

3. Incubate the plate containing the co-culture at 37°C with 5% CO2 for 5-6 hours or overnight.
4. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.
5. Incubate at RT for ~15 to 30 minutes.
6. Measure luminescence using a luminometer.

Notes

To generate a KRAS G12D-Specific 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

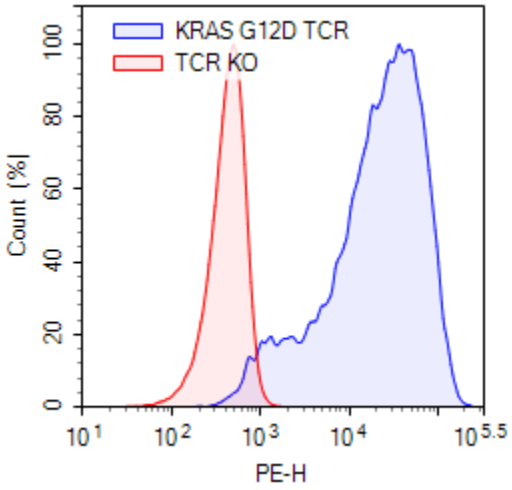

Figure 2. Expression of KRAS G12D-specific TCR in Jurkat cells transduced with the KRAS G12D-Specific TCR Lentivirus (Clone 10).
Approximately 100,000 $CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #78757) were transduced with KRAS G12D-Specific TCR (Clone 10) lentiviruses by spinoculation at a MOI of 10. Sixty-six hours post-transduction, cells were stained with PE anti-mouse TCR β chain Antibody (Biolegend #109207), and the expression of KRAS G12D-specific TCR (clone 10) was analyzed by flow cytometry. The y axis represents the % of cells. The x axis indicates fluorophore intensity.

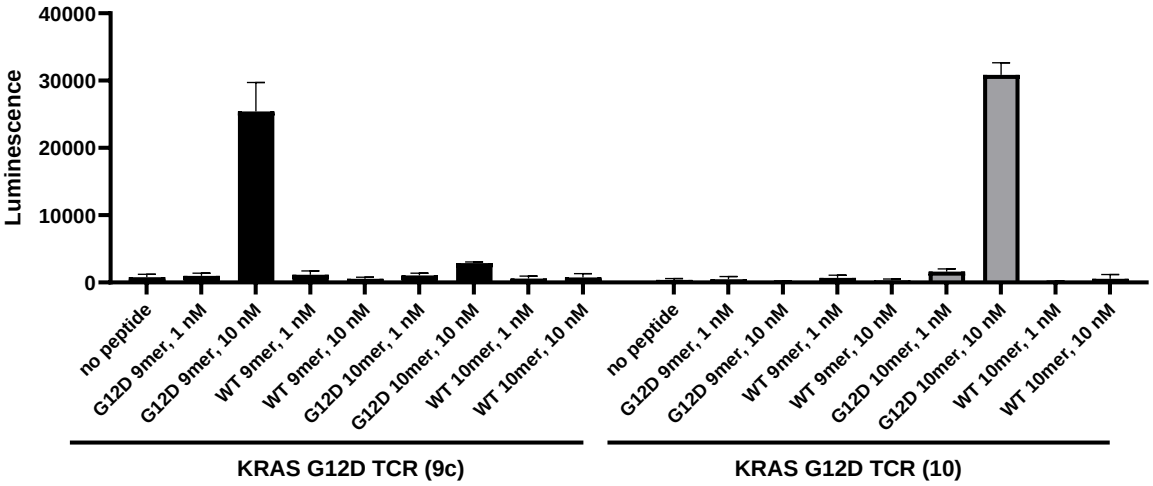

*Figure 3. Jurkat T cell activation after transduction with KRAS G12D -Specific TCR (Clone 10), using HLA-C*08:02 K562 Cell line as antigen-presenting cells (APCs).*
$CD8^+$ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (#78757) were transduced with KRAS G12D TCR Specific Lentivirus (Clone 9c) or KRAS G12D TCR Specific Lentivirus (Clone 10) (#78937) by spinoculation at a MOI of 10. Sixty-six hours post-transduction, the cells were co-cultured overnight with HLA-C*08:02 K562 cells (#78974) loaded with KRAS G12D Peptide 9mer (10-18, #78967), KRAS WT Peptide 9mer (10-18, #78968), KRAS G12D Peptide 10mer (10-19, #78969) or KRAS WT Peptide 10mer (10-19, #78970). Luciferase activity was measured, and the results are shown as raw luminescence readings. Cells transduced with KRAS G12D Specific TCR Lentivirus (Clone 9c) are preferentially activated by KRAS K12D Peptide 9mer, while cells transduced with KRAS G12D Specific TCR Lentivirus (Clone 10) are preferentially activated by KRAS K12D Peptide 10mer. Both KRAS G12D TCR (Clone 9c) and KRAS G12D TCR (Clone 10) transduced cells are specific for mutant KRAS G12D peptides and did not recognize wild type KRAS peptides.

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

References
Eric T., *et al.*, 2016 *N Engl J Med* 375:2255-2262.
Leidner R., *et al.*, 2022 *N Engl J Med* 386:2112-2119.

Related Products

Products	*Catalog #*	*Size*
KRAS G12D Peptide (10-18, 9mer)	78967	100 µl
KRAS Wild Type Peptide (10-18, 9mer)	78968	100 µl
KRAS G12D Peptide (10-19, 10mer)	78969	100 µl
KRAS Wild Type Peptide (10-19, 10mer)	78970	100 µl
KRAS G12D-Specific TCR (Clone 9c) Lentivirus	78936	2 x 500 µl
HLA-C*08:02 K562 Cell line	78974	2 vials

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