Genomeditech (Shanghai) Co.,Ltd.
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Product Sheet

TCR Knockout Reporter Cell Line(CD4+)

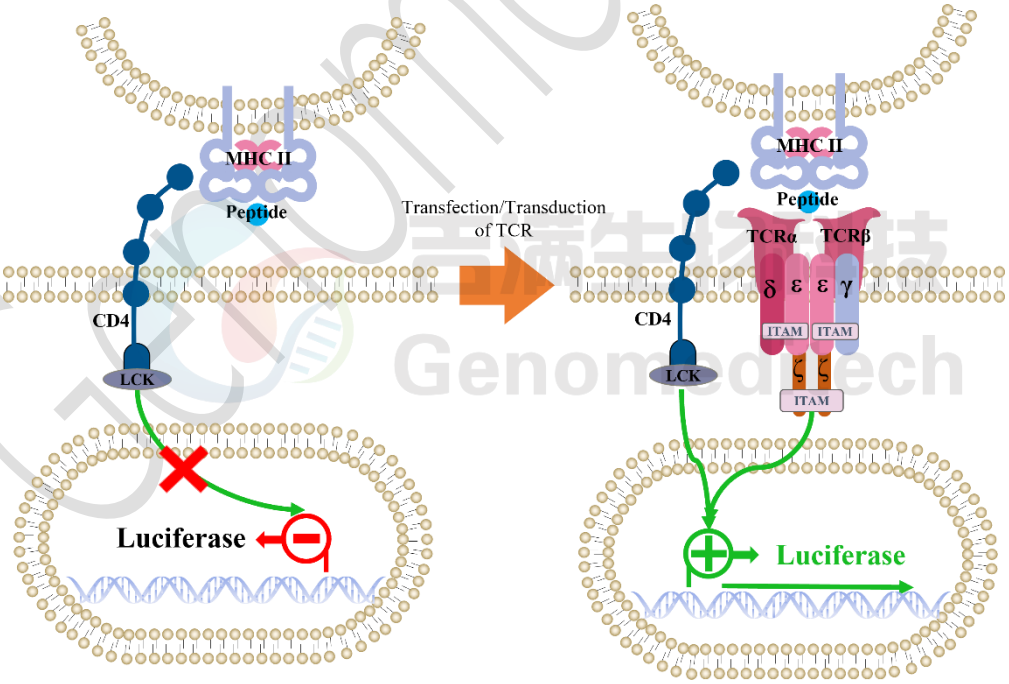
Catalog number: GM-C28018

Version 3.3.1.250116

The T cell receptor (TCR) is crucial for T cells to recognize and bind to antigen peptides presented by major histocompatibility complex (MHC) molecules. CD4 acts as a co-receptor, facilitating T cell recognition and adhesion to MHC class II molecules. Introducing exogenous TCRs enhances T cell's ability to target specific tumor antigens, improving immunotherapy. Sometimes, a patient's T cells may struggle to attack tumor cells effectively; exogenous TCRs can be used to reprogram T cells for greater anti-tumor activity and specificity.

Each T cell has a unique TCR for recognizing various pathogens and tumors. Adding exogenous TCR α and β chains can create a mixed TCR that alters antigen specificity, while mismatches can lessen the effectiveness of the exogenous TCR.

TCR Knockout Reporter Cell Line(CD4+) is a clonal stable cell line constructed using lentiviral technology, knockout endogenously TCR gene, along with signal-dependent expression of a luciferase reporter gene. Researchers can create transgenic TCR cells by introducing a TCR complex, activating them with specific peptides and MHC-expressing APC cells, and measuring luciferase activity afterward. This approach allows for efficient assessment of transgenic TCR efficacy in T cell activation, independent of endogenous TCR interference.



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Specifications

Quantity	5E6 Cells per vial,1 mL
Product Format	1 vial of frozen cells
Shipping	Shipped on dry ice
Storage Conditions	Liquid nitrogen immediately upon receipt
Recovery Medium	RPMI 1640+10% FBS+1% P.S
Growth medium	RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+400 μg/mL G418+200 μg/mL Hygromycin
Note	None
Freezing Medium	90% FBS+10% DMSO
Growth properties	Suspension
Growth Conditions	37°C, 5% CO_2
Mycoplasma Testing	The cell line has been screened to confirm the absence of Mycoplasma species.
Safety considerations	Biosafety Level 2
Note	It is recommended to expand the cell culture and store a minimum of 10 vials at an early passage for potential future use.

Materials

Reagent	Manufacturer/Catalogue No.
RPMI 1640	VivaCell/C3010-0500
Fetal Bovine Serum	Cegrogen biotech/A0500-3010
Pen/Strep	Thermo/15140-122
Blasticidin	Genomeditech/GM-040404
G418	Genomeditech/GM-040402
Hygromycin	Genomeditech/GM-040403
NY-ESO-1 Peptide	GenScript/ RP30225
H_HLA-A*02:01 CHO-K1 Cell Line	In house/
Endogenous TCR Reporter Cell Line(CD4+)	In house/
NY-ESO-1–Specific TCR Reporter Jurkat(TCR KO) Cell Line(CD4+)	In house/
Anti-H_CD4 hIgG1 Antibody(Tregalizumab)	Genomeditech/GM-28752AB
Anti-CD3 epsilon hIgG1 Antibody [OKT-3 (muromonab)]	Genomeditech/GM-51478AB
PE anti-human CD8a Antibody	BioLegend/300907

PE/Cyanine7 anti-human TCR α/β Antibody	BioLegend/306719
GMOne-Step Luciferase Reporter Gene Assay Kit	Genomeditech/GM-040503

Figures

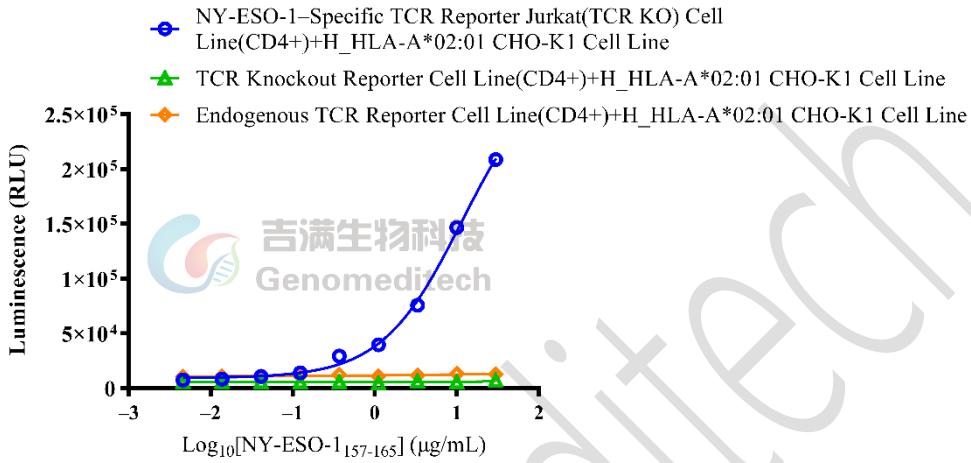

Figure 1 | Response to NY-ESO-1 Peptide. The TCR Knockout Reporter Cell Line(CD4+) (Cat. GM-C28018), NY-ESO-1–Specific TCR Reporter Jurkat(TCR KO) Cell Line(CD4+) and Endogenous TCR Reporter Cell Line(CD4+) at a concentration of 1E5 cells/well were co-cultured with H_HLA-A*02:01 CHO-K1 Cell Line at a concentration of 1.5E4 cells/well, in the presence of serial dilutions of the NY-ESO-1 Peptide (GenScript/ RP30225) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S) for 6 hours (96-well format). The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.

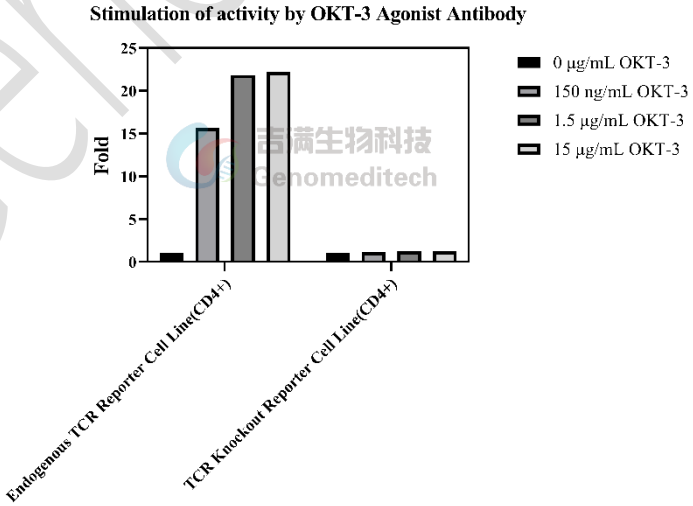

Figure 2 | Response to Anti-CD3 epsilon Antibody [OKT-3 (muromonab)]. The TCR Knockout Reporter Cell Line(CD4+) (Cat. GM-C28018) and Endogenous TCR Reporter Cell Line(CD4+) at a concentration of 1E5 cells/well (96-well format) was stimulated with serial dilutions of Anti-CD3 epsilon Antibody [OKT-3 (muromonab)] (Cat. GM-

51478AB) in assay buffer (RPMI 1640 + 1% FBS + 1% P.S). The firefly luciferase activity was measured using the GMOne-Step Luciferase Reporter Gene Assay Kit (Cat. GM-040503). Data are shown by drug mass concentration.

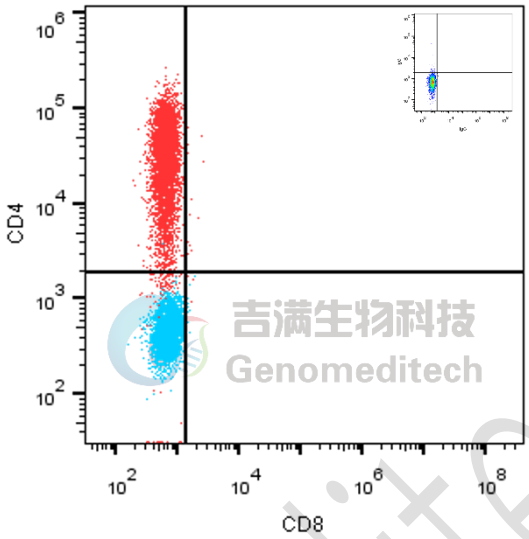

Figure 3 | TCR Knockout Reporter Cell Line(CD4+) (Cat. GM-C28018) was determined by flow cytometry using Anti-H_CD4 hIgG1 Antibody(Tregalizumab) (Cat. GM-28752AB) and PE anti-human CD8a Antibody (BioLegend/300907).

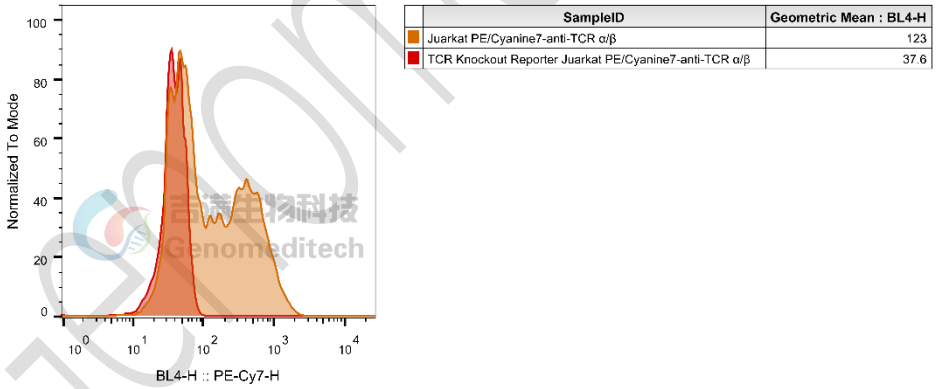

Figure 4 | TCR Knockout Reporter Cell Line(CD4+) (Cat. GM-C28018) was determined by flow cytometry using PE/Cyanine7 anti-human TCR α/β Antibody (BioLegend/306719).

Cell Recovery

Recovery Medium: RPMI 1640+10% FBS+1% P.S

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To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

a) Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 - 3 minutes).
b) Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
c) Transfer the vial contents to a centrifuge tube containing 5.0 mL complete culture medium. And spin at approximately 176 x g for 5 minutes. Discard supernatant.
d) Resuspend cell pellet with the recommended complete medium. And dispense the suspension into 1 - 2 T-25 culture flasks.
e) Incubate the culture at 37°C in a suitable incubator. A 5% CO_2 in air atmosphere is recommended if using the medium described on this product sheet.

Cell Freezing

Freezing Medium: 90% FBS+10% DMSO

a) Centrifuge at 176 x g for 3 minutes to collect cells.
b) Resuspend the cells in pre-cooled freezing medium and adjust the cell density to 5E6 cells/mL.
c) Aliquot 1 mL into each vial.
d) Place the vial in a controlled-rate freezing container and store at -80°C for at least 1 day, then transfer to liquid nitrogen as soon as possible.

Cell passage

Growth medium: RPMI 1640+10% FBS+1% P.S+3.5 μg/mL Blasticidin+400 μg/mL G418+200 μg/mL Hygromycin

Approximately 48-72 hours after the initial thawing, the cells can be passaged for the first time. After this initial passage, the culture medium can be adjusted to growth medium supplemented with antibiotics. If cells are not passaged within 48 hours, it is recommended to add some fresh recovery medium and place the flask horizontally.

a) When the cell density reaches 1.5 - 2E6 cells/mL, subculture the cells. Do not allow the cell density to exceed 2E6 cells/mL.
b) It is recommended to use T-25 flasks for subculturing.
c) These cells are suspension cells, and it is recommended to use the "half-medium change" method to maintain optimal cell conditions during passaging.
d) During passaging, you can directly add fresh growth medium to the culture flask, gently pipette to resuspend the cells, and then transfer the cell suspension to a new T-25 flask for continued culture.

Subcultivation Ratio: Maintain cultures at a cell concentraion between 3E5 and 1E6 viable cells/mL.

Medium Renewal: Every 2 to 3 days

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Notes

a) These cells are sensitive to density, so please ensure that the cell density is maintained within an appropriate range during culture and subculturing.

b) During the first passage, pay attention to the nutrient supply; if not subculturing, make sure to add fresh recovery medium every other day as needed.

Related Products

TCR	
H_FOXP3-Promoter Reporter Jurkat Cell Line	H_IL2-Promoter Reporter Jurkat Cell Line
NFAT-Luc Reporter Jurkat Cell Line	OKT3(CD3 ScFv) CHO-K1 Cell Line
Anti-CD3-CD19 Bispecific Antibody(Blinatumomab)	

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