Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

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Catalog No.	Size
CJUR-STK269	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) was engineered to not only express the NFAT signaling response element and the full length human CD8A (Uniprot: P01732) and CD8B (Uniprot: P10966), but also targeted knockout of human TRAC(Gene ID: 28755), TRBC1 (Gene ID: 28639)，TRBC2 (Gene ID: 28638) and CD4 (Gene ID: 920), The expression level of human TCRαβ，CD4 and CD8 were confirmed by flow cytometry. Mutated sequences of human TCRαβ and CD4 produced by nonhomologous end joining (NHEJ) were confirmed through genomic sequencing. This reporter cell can be equipped with either a chimeric antigen receptor (CAR) or a T cell receptor (TCR), providing a cell-based functional platform for CAR or TCR screening and characterization by detecting the change in luminescence.

• *Application*

- Screen for CAR.
- Screen for MHC I restricted antigen-specific TCR.

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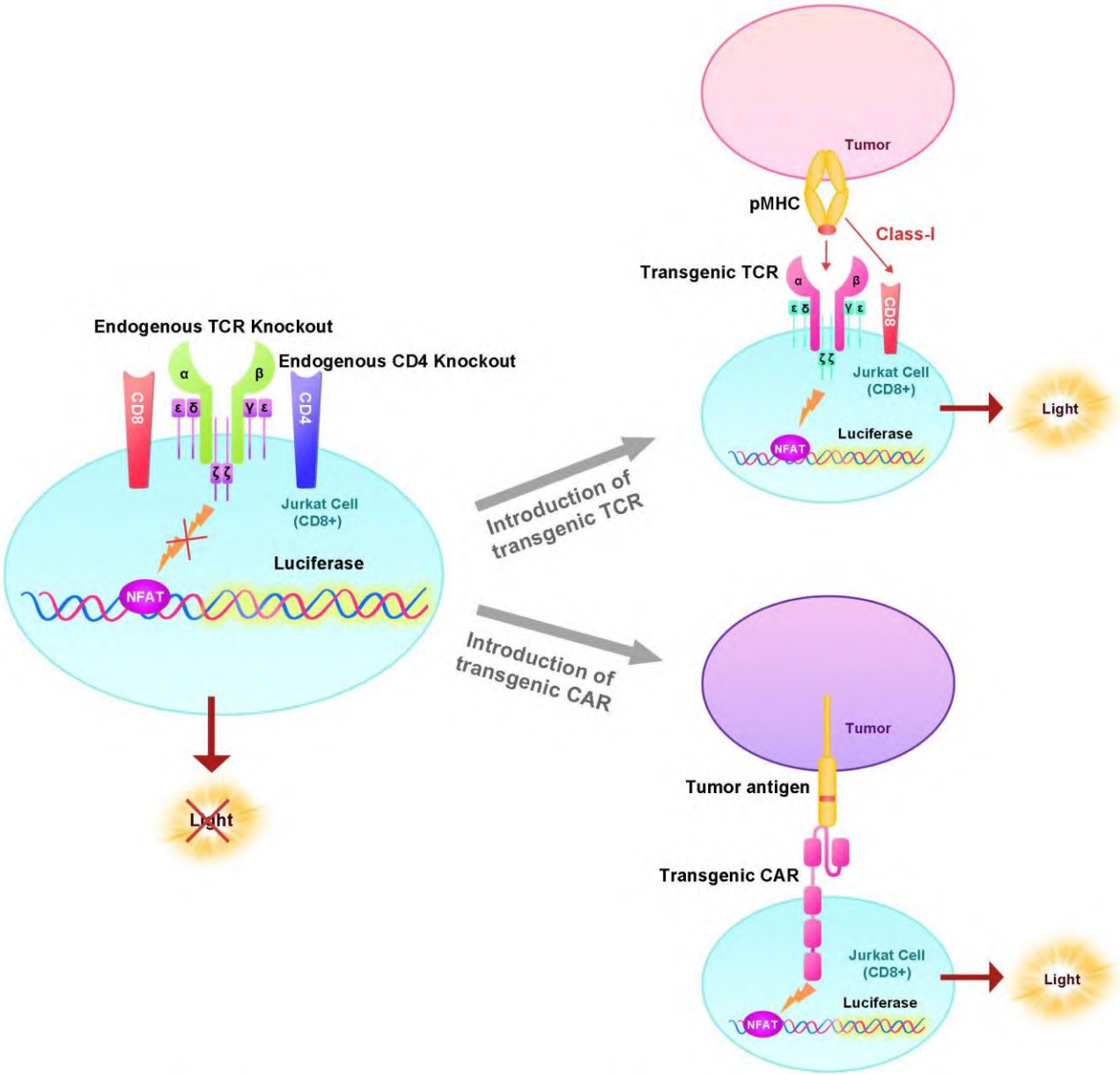
- ***Application principle***



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• *Cell Line Profile*

Cell line	Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+)
Host Cell	Jurkat
Property	Suspension
Complete Growth Medium	RPMI-1640 + 10% FBS
Selection Marker	Puromycin (5 μg/mL) + Hygromycin B (20 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	16-20 hours

• *Materials Required for Cell Culture*

- RPMI Medium 1640 (Gibco, Cat. No. 11875-093)
- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)
- Hygromycin B (Invitrogen, Cat. No. 10687010)

Note: For selection antibiotics, we highly recommend using the specified brand. The activity of antibiotics may vary between manufacturers, so if you choose to use a different brand, it is essential to validate whether the concentration recommended in the culture medium is suitable. Regardless of the brand used, we recommend maintaining a backup culture without selection antibiotics to avoid potential cell loss due to inappropriate antibiotic concentration.

- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Complete Growth Medium: RPMI-1640 + 10% FBS, 1%P/S
- Culture Medium: RPMI-1640 + 10% FBS, Puromycin (5 μg/mL), Hygromycin B (20 μg/mL), 1%P/S
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, Cat. No. 430641)
- Cryogenic storage vials (SARSTEDT, Cat. No. 72.379.007)
- Thermostat water bath
- Centrifuge (Cence, Model: L550)
- Cell counter (MONWEI, Model: SmartCell200A Plus)
- CO2 Incubator (Thermo, Model: 3111)
- Biological Safety Cabinet (Thermo, Model: 1389)

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Recovery*

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize the risk of contamination, ensure the cap remains out of the water. Thawing should be completed quickly, typically within 3-5 minutes.
2. After thawing, promptly remove the vial from the water bath and decontaminate it by spraying with 70% ethanol. From this point onward, all operations must be performed under strict aseptic conditions.
3. Transfer the contents of the vial to a centrifuge tube containing 4.0 mL of complete growth medium.
4. Count viable cells and centrifuge at approximately 1000 rpm for 5 minutes.
5. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh complete growth medium. Adjust the cell density of the suspension to 1×10^6 viable cells/mL and transfer cells to an appropriate size vessel.
6. Incubate at 37°C with 5% CO_2 incubator.

• *Subculture*

Cell viability may be low after thawing, and full recovery (viability >90%) may take up to 1-2 weeks. Once the cell density reaches approximately 2×10^6 viable cells/mL, adjust the density to a range of 2×10^5-5×10^5 viable cells/mL by either adding the fresh culture medium or replacing the existing culture medium. Avoid allowing the cell density to exceed 3×10^6 cells/mL, as this may negatively impact cell performance in subsequent passages. T-75 flasks are recommended for subculturing.

• **Subculturing Frequency:** It is recommended to subculture every 3-4 days, adjusting the frequency based on the cell density in your specific culture system.

Note: After recovery, maintain the cells for 1-2 passages in the complete growth medium not containing the selection marker, if the cells are in good condition (viability >90%), transition to the culture medium containing the selection marker during subculturing.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Cryopreservation*

1. Count viable cells and harvest the cell suspension.
2. Centrifuge at 1000 rpm for 5 min at room temperature and resuspend cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
3. Aliquot the cell suspension into cryogenic storage vials. Place the vials in a programmable cooler or an insulated box placed in a –80°C freezer overnight, then transfer to liquid nitrogen storage for long-term storage.

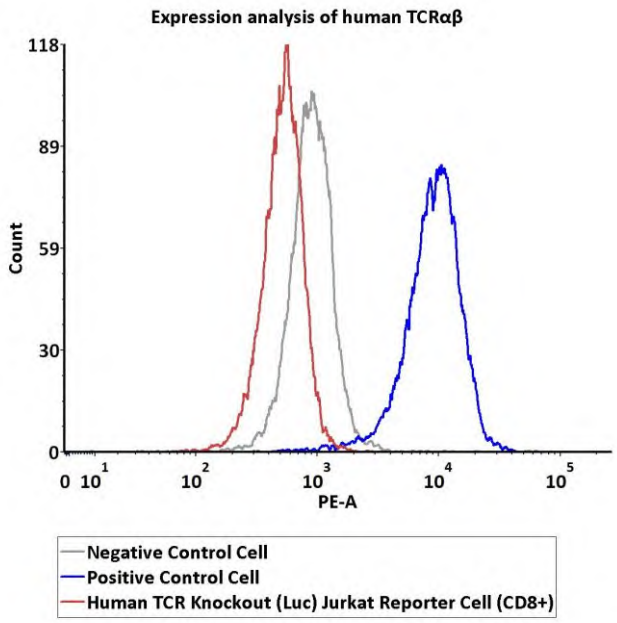
Note: It is recommended to establish a cell bank at the earliest possible passage for long-term use.

• *Storage*

Cells must be received in a frozen state on dry ice and should be transferred to liquid nitrogen or a –80°C freezer immediately upon receipt. If stored in a –80°C freezer, it is recommended to limit the storage period to no more than two weeks. For long-term preservation, transfer the cells to liquid nitrogen is highly recommended.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+ CD8+) Data Sheet

• *Receptor Assay*



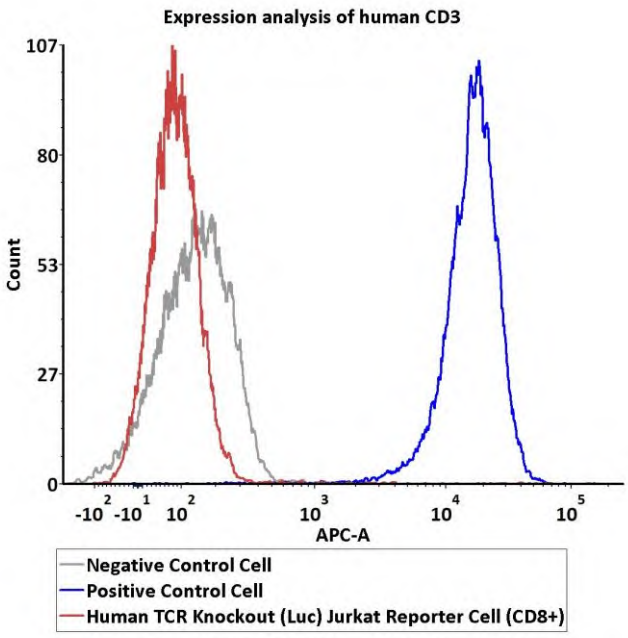
Catalog No.	Stable Cell Line	MFI for TCRαβ (PE)
NA	Negative Control Cell	877.58
NA	Positive Control Cell	9132.02
CJUR-STK269	Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+)	518.17

Fig1. Expression analysis of human TCRαβ on Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) by FACS.

Cell surface staining was performed on Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) using PE-labeled anti-human TCRαβ antibody. The parental NFAT (Luc) Jurkat Reporter Cell (Cat. No. SCJUR-STF046) was stained with PE-labeled anti-human TCRαβ antibody as the positive control cell. The parental NFAT (Luc) Jurkat Reporter Cell was stained with PE-labeled isotype control antibody as the negative control cell.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Receptor Assay*



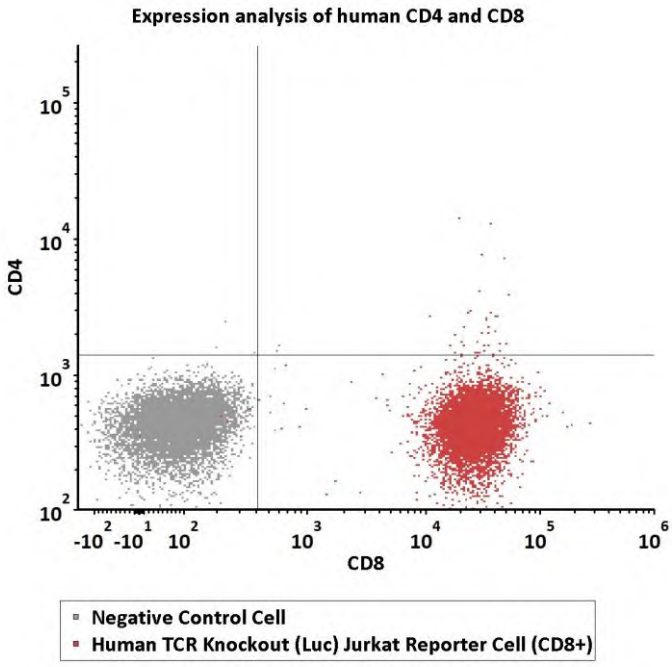
Catalog No.	Stable Cell Line	MFI for CD3 (APC)
NA	Negative Control Cell	134.53
NA	Positive Control Cell	16142.00
CJUR-STK269	Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+)	79.26

Fig2. Expression analysis of human CD3 on Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) by FACS.

Cell surface staining was performed on Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) using APC-labeled anti-human CD3 antibody. The parental NFAT (Luc) Jurkat Reporter Cell (Cat. No. SCJUR-STF046) was stained with APC-labeled anti-human CD3 antibody as the positive control cell. The parental NFAT (Luc) Jurkat Reporter Cell was stained with APC-labeled isotype control antibody as the negative control cell.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

- *Receptor Assay*



Catalog No.	Stable Cell Line	MFI for CD4 (PE)	MFI for CD8 (APC)
NA	Negative Control Cell	517.18	89
CJUR-STK269	Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+)	455.07	26312.51

Fig3. Expression analysis of human CD4 and human CD8 on Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) by FACS.

Cell surface staining was performed on Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) using PE-labeled anti-human CD4 antibody and APC-labeled anti-human CD8 antibody. The Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) was stained with PE-labeled isotype control antibody and APC-labeled isotype control antibody as the negative control cell.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Sequencing Analysis*

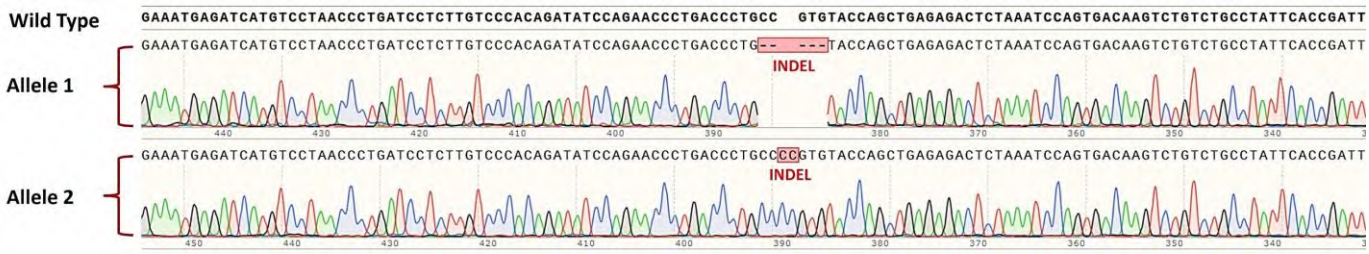


Fig4. Genomic Sequencing of human TRAC in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

Sanger sequencing was used for mutation analysis of human TRAC. The sequencing results demonstrated that frameshift mutations were generated in the human TRAC gene in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

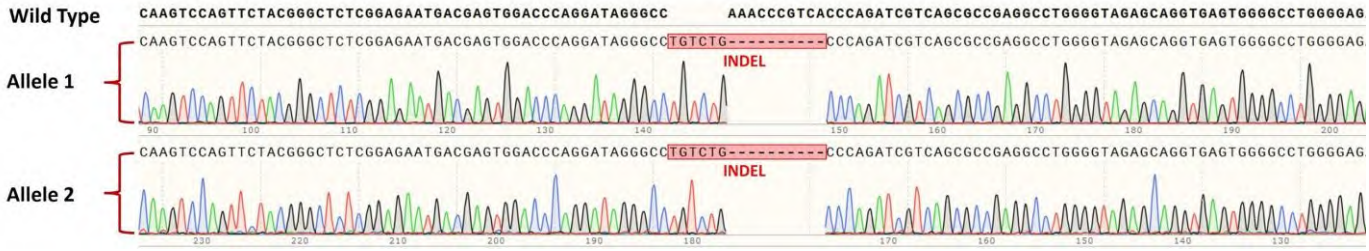


Fig5. Genomic Sequencing of human TRBC1 in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

Sanger sequencing was used for mutation analysis of human TRBC1. The sequencing results demonstrated that frameshift mutations were generated in the human TRBC1 gene in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

- ***Sequencing Analysis***

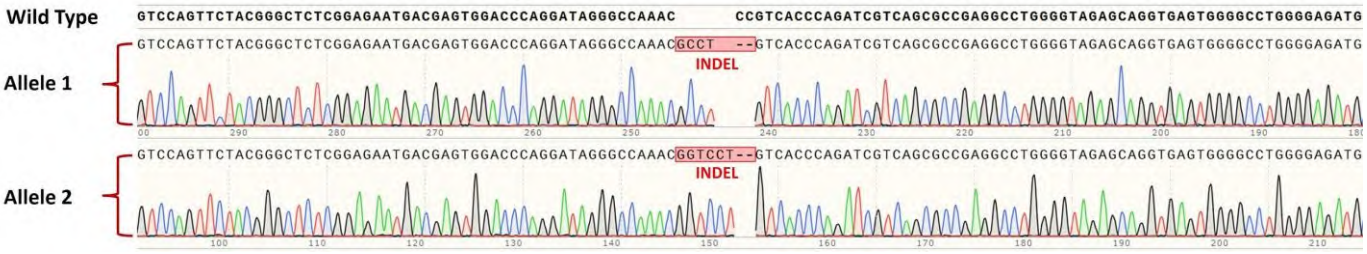


Fig6. Genomic Sequencing of human TRBC2 in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

Sanger sequencing was used for mutation analysis of human TRBC2. The sequencing results demonstrated that frameshift mutations were generated in the human TRBC2 gene in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

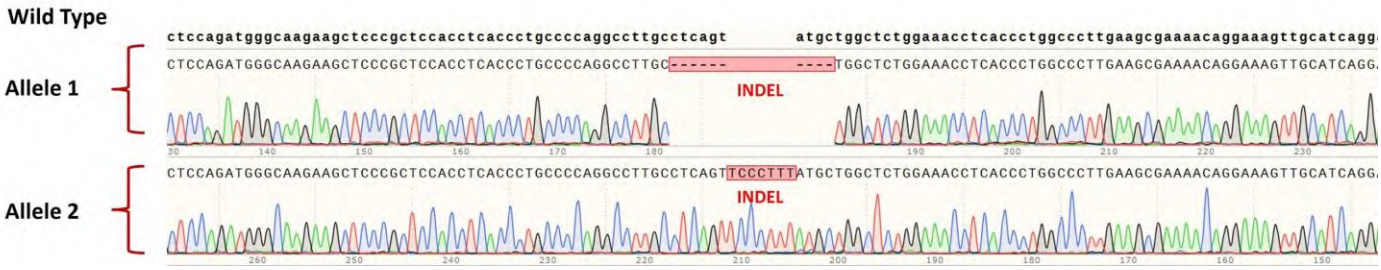


Fig7. Genomic Sequencing of human CD4 in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

Sanger sequencing was used for mutation analysis of human CD4. The sequencing results demonstrated that frameshift mutations were generated in the human CD4 gene in the Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+).

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Signaling Bioassay*

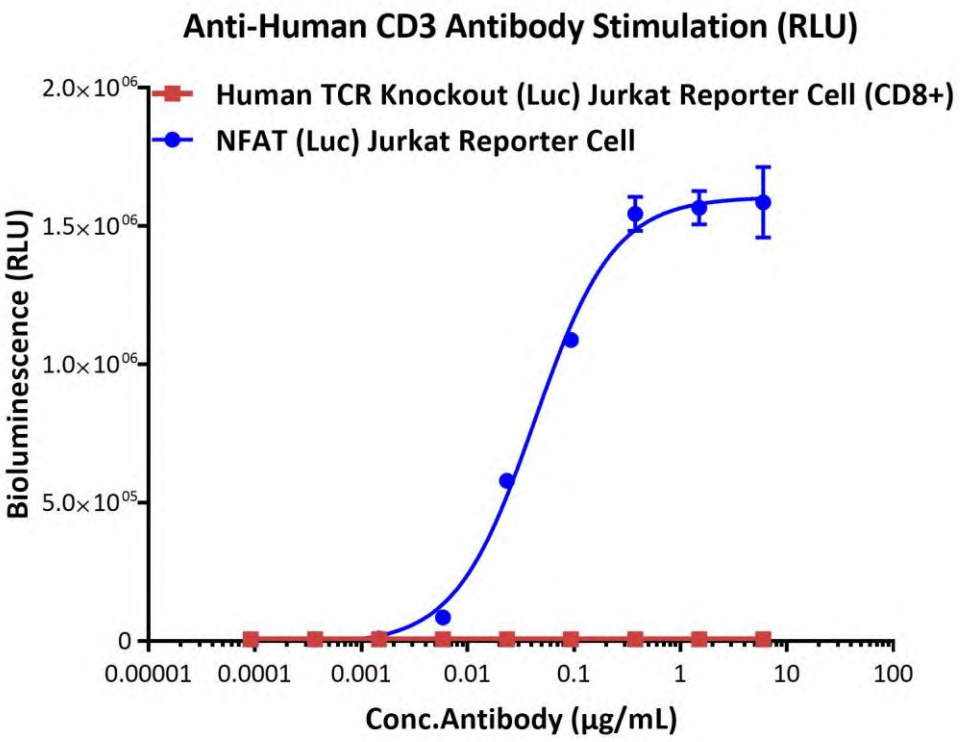


Fig8. Response to anti-human CD3 antibody (RLU).

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) and the parental NFAT (Luc) Jurkat Reporter Cell (Cat. No. SCJUR-STF046) were incubated with serial dilutions of anti-human CD3 antibody (Cat. No. CDE-M120a). Compared to the parental NFAT (Luc) Jurkat Reporter Cell, Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) exhibited no activation response to anti-human CD3 antibody stimulation.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Signaling Bioassay*

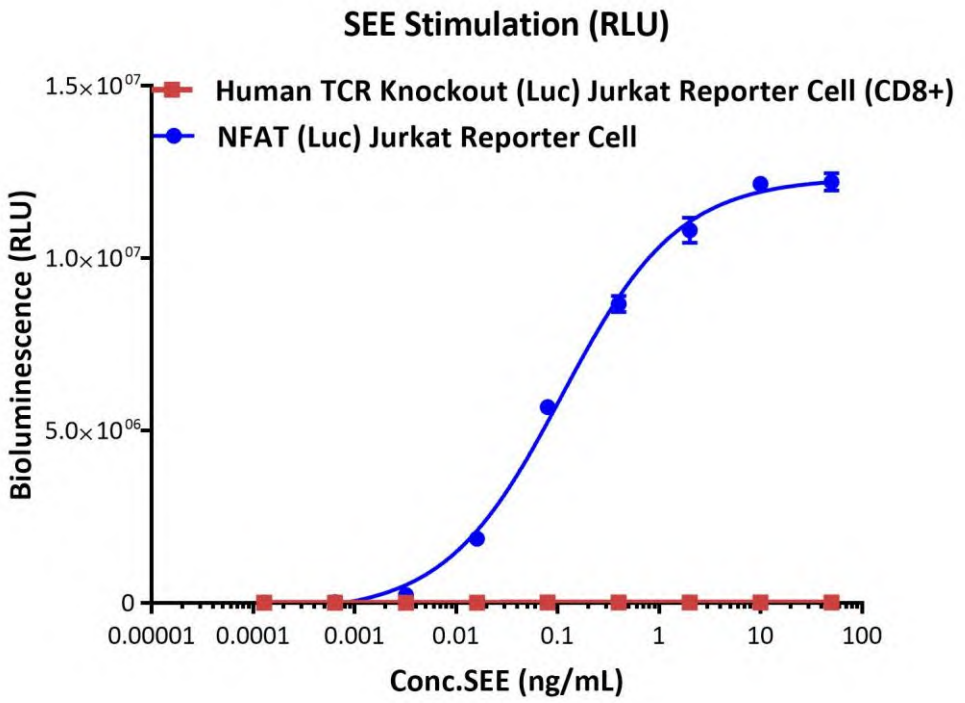


Fig9. Response to SEE (RLU).

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) and the parental NFAT (Luc) Jurkat Reporter Cell (Cat. No. SCJUR-STF046) were incubated with serial dilutions of SEE in the presence of target cells expressing MHC II. Compared to the parental NFAT (Luc) Jurkat Reporter Cell, Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) exhibited no activation response to SEE stimulation in the presence of target cells expressing MHC II.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Signaling Bioassay*

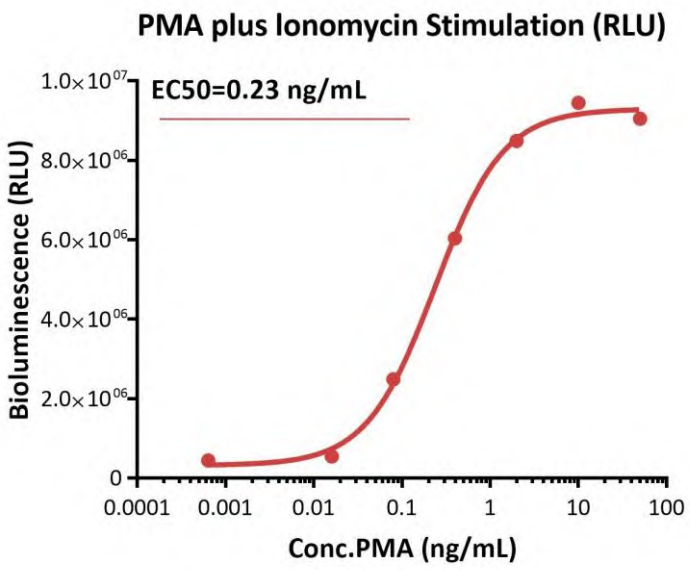


Fig10. Response to PMA plus Ionomycin (RLU).

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) was stimulated with serial dilutions of PMA plus Ionomycin (1 μM). The EC50 was approximately 0.23ng/mL.

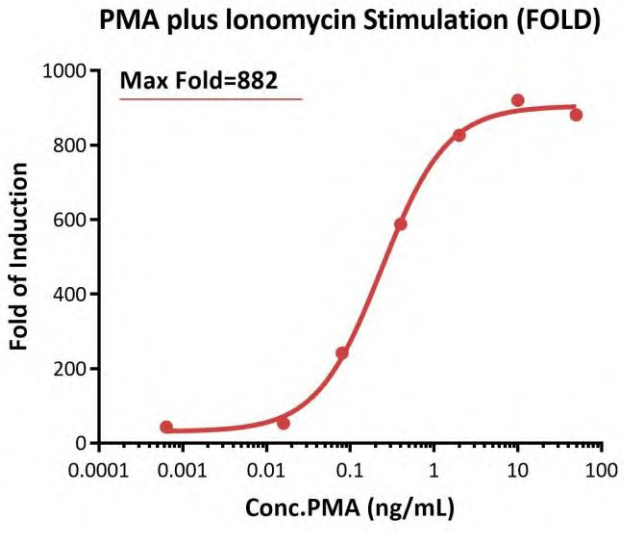


Fig11. Response to PMA plus Ionomycin (FOLD).

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) was stimulated with serial dilutions of PMA plus Ionomycin (1 μM). The max induction fold was approximately 882.

Human TCR Knockout (Luc) Jurkat Reporter Cell (CD8+) Data Sheet

• *Related Products*

Products	Cat.No.
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+)	CJUR-STF247
Human TCR Knockout (Luc) Jurkat Reporter Cell (CD4+ CD8+)	CJUR-STK270
Raji/Human CD19 Knockout Stable Cell Line	SCRAJ-STT216
Raji/Human CD20 Knockout Stable Cell Line	SCRAJ-STT227
Raji/Human CD19 & CD20 Double Knockout Stable Cell Line	CRAJ-STK238
Jurkat/Human TRBV9 & Luc Stable Cell Line	CJUR-STP292
Jurkat/Human TRBV9 Stable Cell Line	CJUR-STP293
Raji/Luc & Human CD19 Knockout Stable Cell Line	CRAJ-STK286
Raji/Luc & Human CD20 Knockout Stable Cell Line	CRAJ-STK285
Raji/Luc & Human CD19 & CD20 Double Knockout Stable Cell Line	CRAJ-STK284
NFAT (Luc) Jurkat Reporter Cell	SCJUR-STF046
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
Human EGF R (Luc) HEK293 Reporter Cell	CHEK-ATF049
NFAT (Luc) HEK293 Reporter Cell	CHEK-ATF050
HEK293/Human CCR5 Stable Cell Line	CHEK-ATP043
HEK293/Human SIRP alpha Stable Cell Line	CHEK-ATP051
HEK293/Human CD20 Stable Cell Line	CHEK-ATP034
HEK293/Human ASGR1 Stable Cell Line	CHEK-ATP080
HEK293/Human TMPRSS2-HA-P2A-mGFP Stable Cell Line	CHEK-ATP101
NF-kB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
NY-ESO-1 specific TCR-HEK293 cell line	CHEK-STP114
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
HEK293/Human CCR8 Stable Cell Line	CHEK-ATP140
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144

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• *Related Products*

Products	Cat.No.
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
Human HVEM (Luc) HEK293 Reporter Cell	CHEK-ATF105
Human BTLA (Luc) Jurkat Reporter Cell	SCJUR-STF106
Human IGF-1 R (Luc) HEK293 Reporter Cell	CHEK-ATF107
NF-kB (Luc) Jurkat Reporter Cell	SCJUR-STF113
TCF/LEF (Luc) HEK293 Reporter Cell	CHEK-ATF114
Human GLP-2R (Luc) HEK293 Reporter Cell	CHEK-ATF128
Human 5-HT1A (Luc) HEK293 Reporter Cell	CHEK-ATF131
Human RANK (Luc) HEK293 Reporter Cell	CHEK-ATF129
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130
Human IL-17 RA/IL-17 RC (Luc) HEK293 Reporter Cell	CHEK-ATF133
ISRE (Luc) HEK293 Reporter Cell	CHEK-ATF134
Human OX40 (Luc) HEK293 Reporter Cell	CHEK-ATF135
Human IL-2 R beta/IL-2 R gamma (Luc) HEK293 Reporter Cell	CHEK-ATF136
Human c-MET (Luc) HEK293 Reporter Cell	CHEK-ATF144
Human TGF-beta R (Luc) HEK293 Reporter Cell	CHEK-ATF145
Human FGF-21 (Luc) HEK293 Reporter Cell	CHEK-ATF163
Human Activin RII (Luc) HEK293 Reporter Cell	CHEK-ATF164
Human IL-23 R/IL-12 R beta 1(Luc) HEK293 Reporter Cell	CHEK-ATF166
Human IL-22 R alpha 1/IL-10 R beta (Luc) HEK293 Reporter Cell	CHEK-ATF167
Human VEGF R2 (Luc) HEK293 Reporter Cell	CHEK-ATF044
Human TSLPR (Luc) HEK293 Reporter Cell	CHEK-ATF045
Human VEGF R2 (Luc) HEK293 Reporter Cell	CHEK-ATF044
STAT3 (Luc) HEK293 Reporter Cell	CHEK-ATF047
NF-κB (Luc) HEK293 Reporter Cell	CHEK-ATF048
Human EGF R (Luc) HEK293 Reporter Cell	CHEK-ATF049