NFAT (Luc) Jurkat Reporter Cell Development Service Data Sheet

NFAT (Luc) Jurkat Reporter Cell

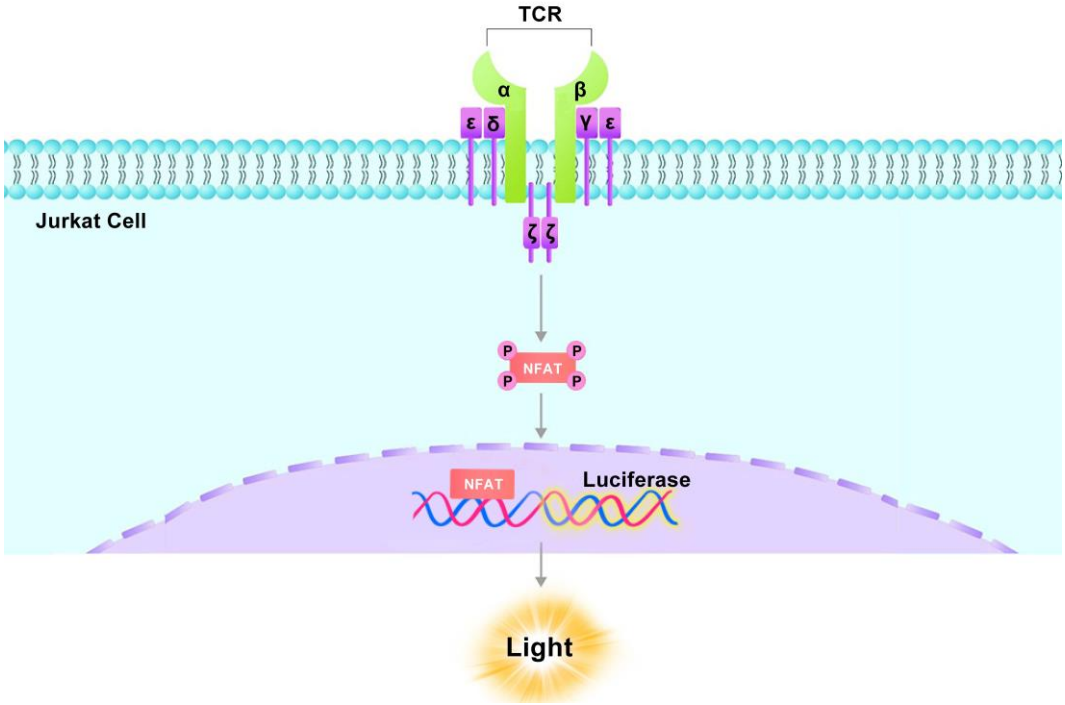
Catalog No.	Size
SCJUR-STF046	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The NFAT (Luc) Jurkat Reporter Cell was engineered with the NFAT response element driving luciferase expressing systems. We could equip this reporter cell with a chimeric antigen receptor (CAR) for developing a CAR-J-based activity screening system. The anti-TCR/CD3 inducing intracellular signals could be inhibited by some transfected immune checkpoints binding to corresponding ligands.

• *Application*

- Transfection host for some immune checkpoint concerning the NFAT signaling pathway
- The discovery of T cell activators by the NFAT signaling bioactivity
- Screen for anti-human CD3xTAA BsAb



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• *Cell Line Profile*

Cell line	NFAT (Luc) Jurkat Reporter Cell
Host Cell	Jurkat
Property	Suspension
Complete Growth Medium	RPMI-1640 + 10% FBS
Selection Marker	Puromycin (5 μg/mL)
Incubation	37℃ with 5% CO_2
Doubling Time	16-20 hours
Transduction Technique	Lentivirus

• *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)
- Complete Growth Medium: RPMI-1640 + 10% FBS
- Culture Medium: RPMI-1640 + 10% FBS, Puromycin (5 μg/mL)
- Freeze Medium: 90% FBS, 10% (V/V) DMSO
- T-75 Culture flask (Corning, 430641)
- Cryogenic storage vials (SARSTEDT, 72.379.007)
- Thermostat water bath
- Centrifuge
- Luna cell counter (Logos Biosystems, LUNA-Ⅱ)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

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• *Recovery*

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the cap out of the water. Thawing should be rapid (approximately 5 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by spraying with 70% ethanol. All the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer the vial contents to a centrifuge tube containing 4.0 mL complete growth medium.
4. Count viable cells and spin 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℃ with 5% CO_2 incubator

• *Subculture*

Adjust the cell density at 2×10^5-5×10^5 viable cells/mL by the addition of fresh culture medium or replacement of culture medium. Do not allow the cell density to exceed 3×10^6 cells/mL. T-75 flasks are recommended for subculturing.

- **Medium Renewal:** Add fresh culture medium every 3 to 4 days (depending on cell density)

• *Cryopreservation*

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

• *Storage*

- **Product format:** Frozen
- **Storage conditions:** Liquid nitrogen immediately upon receipt

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• *Receptor Assay*

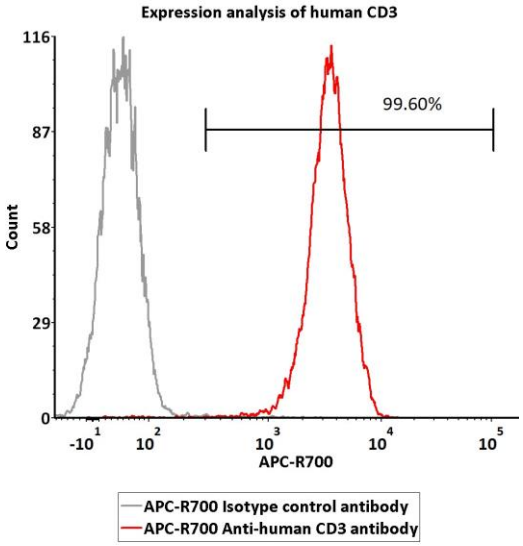

Fig1. Expression analysis of human CD3 on NFAT (Luc) Jurkat Reporter Cell by FACS. NFAT (Luc) Jurkat Reporter Cell were stained with APC-R700 labeled Anti-Human CD3 antibody or APC-R700 labeled Isotype antibody.

• *Application*

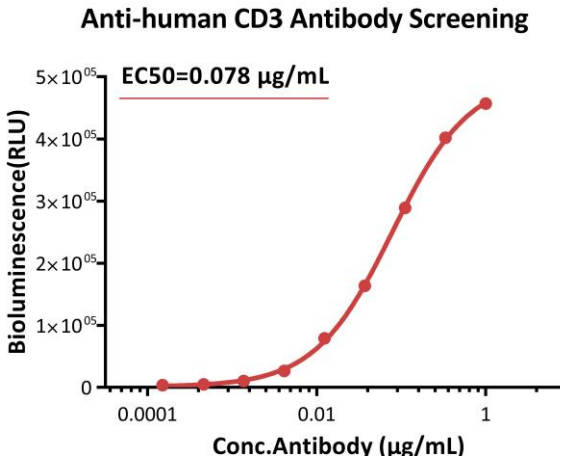

Fig2. Activating of NFAT signaling bioactivity by anti-human CD3 antibody (RLU)**.** This reporter cell was incubated with serial dilutions of anti-human CD3 antibody (AcroBiosystems, Cat.No.CDE-M120a). The EC50 of anti-human CD3 antibody was approximately 0.078 μg/mL.

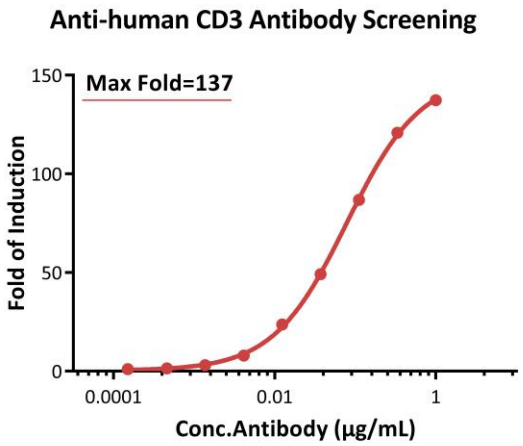

Fig3. Activating of NFAT signaling bioactivity by anti-human CD3 antibody (Fold). This reporter cell was incubated with serial dilutions of anti-human CD3 antibody (AcroBiosystems, Cat.No.CDE-M120a). The max induction fold was approximately 137.

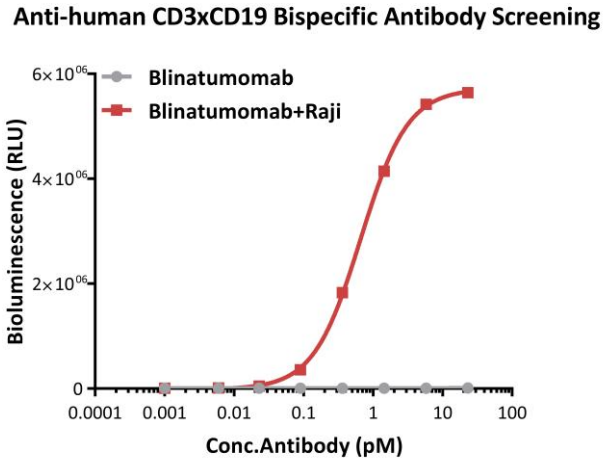

Fig4. Bioactivity detection of Anti-human CD3xCD19 bispecific antibody. This reporter cell was incubated with serial dilutions of Blinatumomab (CD3×CD19 BsAb) in the presence of Raji cells that express human CD19 endogenously. The EC50 of Blinatumomab incubated with Raji cells is approximately 0.66 pM with the max induction fold 842.

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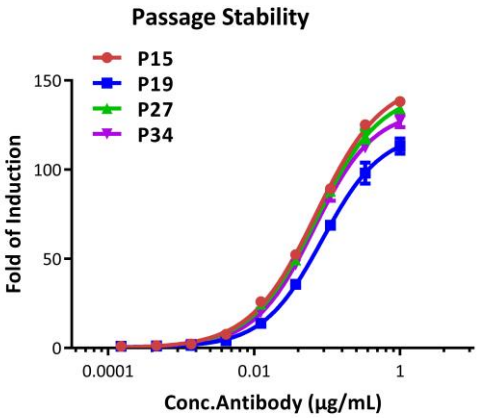
- ***Passage Stability***



	P15	P19	P27	P34
EC50 (μg/mL)	0.071	0.086	0.071	0.066
Max Fold	138	113	134	127

Fig5. Passage stability analysis by anti-human CD3 antibody stimulation. The continuously growing NFAT (Luc) Jurkat Reporter Cell was stimulated with serial dilutions of anti-human CD3 antibody (AcroBiosystems, Cat.No.CDE-M120a). Anti-human CD3 antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 15-34.

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• *License Disclosure*

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• *Related Products*

Products	**Cat.No.**
Human CD16a (158V) (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF067
Human CD32a (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF069
Human CD32a (131R) (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF070
Human CD32b (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF071
Human CD64 (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF072