NFAT (Luc) Jurkat Reporter Cell Data Sheet

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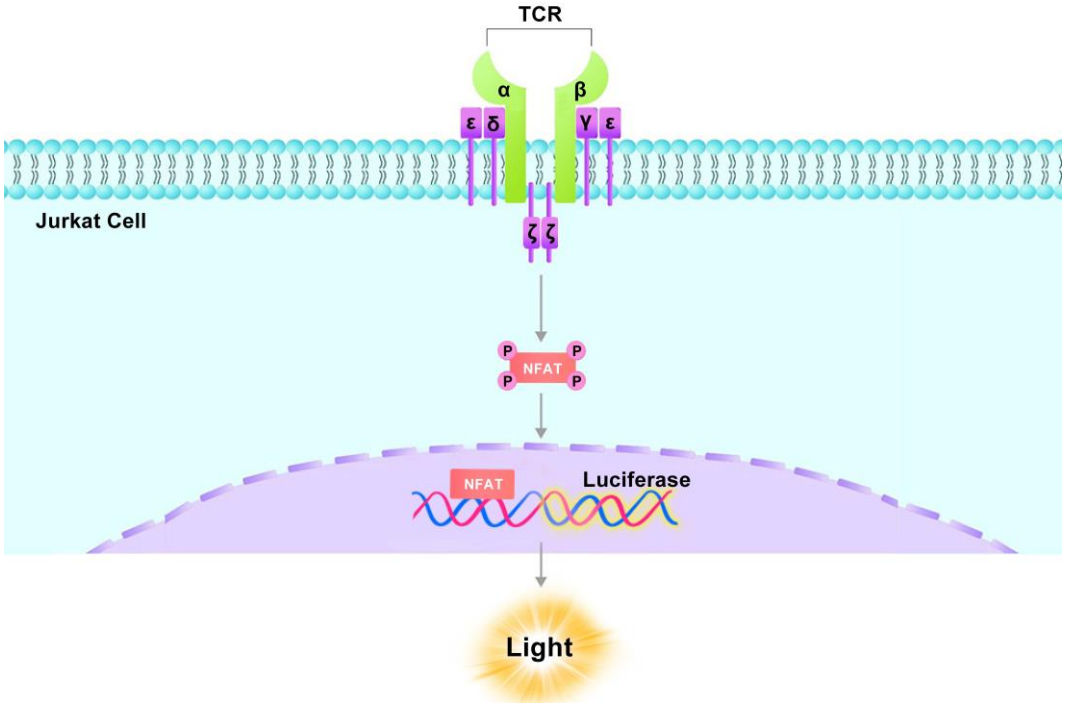
Catalog No.	Size
SCJUR-STF046	2 × (1 vial contains ~5×10^6 cells)

• *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°C 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)

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), 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)

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• *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℃ 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.

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• *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.

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• *Receptor Assay*

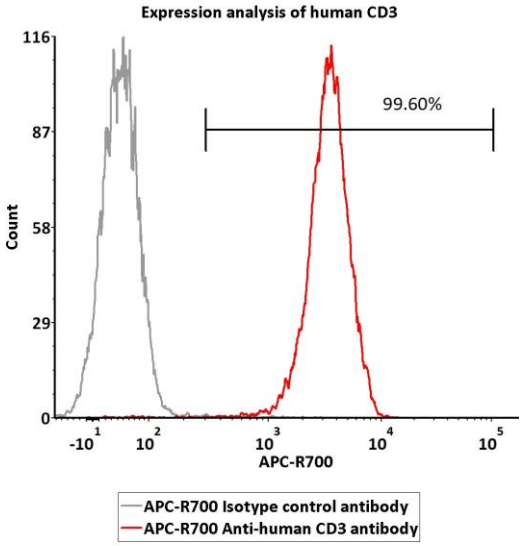

Fig1. Expression analysis of human CD3 on NFAT (Luc) Jurkat Reporter Cell by FACS. NFAT (Luc) Jurkat Reporter Cell were stained with APC-R700 labeled Anti-Human CD3 antibody or APC-R700 labeled Isotype antibody.

• *Application*

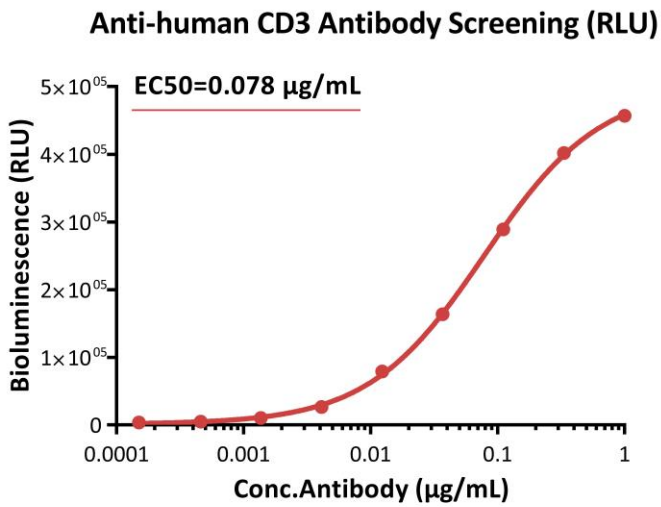

Fig2. Activating of NFAT signaling bioactivity by anti-human CD3 antibody (RLU). This reporter cell was incubated with serial dilutions of anti-human CD3 antibody (Cat. No. CDE-M120a). The EC50 of anti-human CD3 antibody was approximately 0.078 μg/mL.

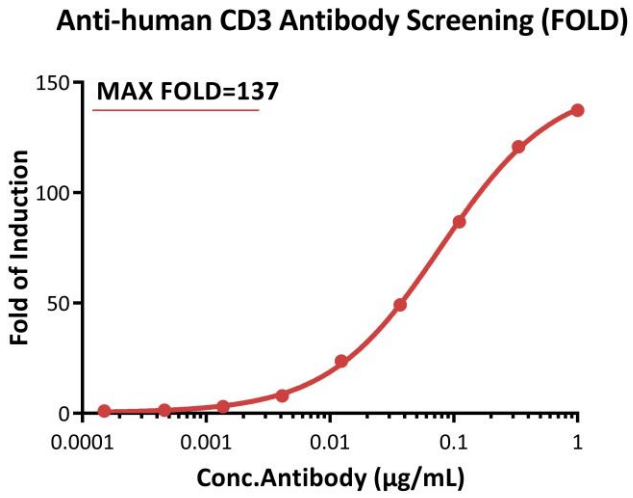

Fig3. Activating of NFAT signaling bioactivity by anti-human CD3 antibody (FOLD). This reporter cell was incubated with serial dilutions of anti-human CD3 antibody (Cat. No. CDE-M120a). The max induction fold was approximately 137.

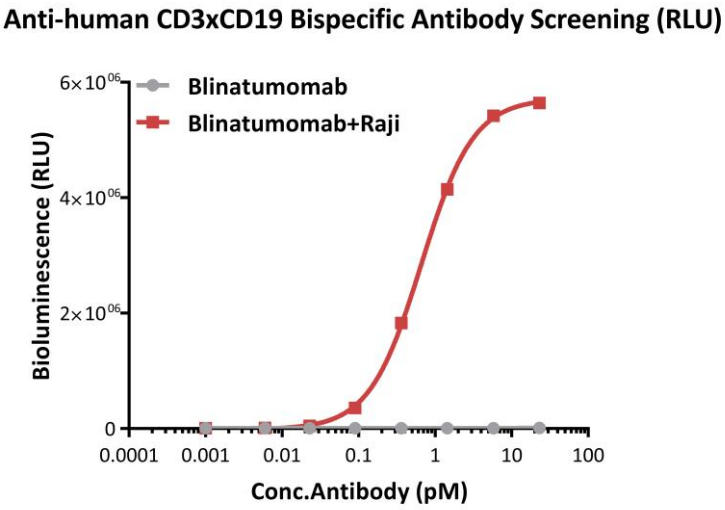

Fig4. Bioactivity detection of Anti-human CD3xCD19 bispecific antibody. This reporter cell was incubated with serial dilutions of Blinatumomab (CD3×CD19 BsAb) in the presence of Raji cells that express human CD19 endogenously. The EC50 of Blinatumomab incubated with Raji cells is approximately 0.66 pM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

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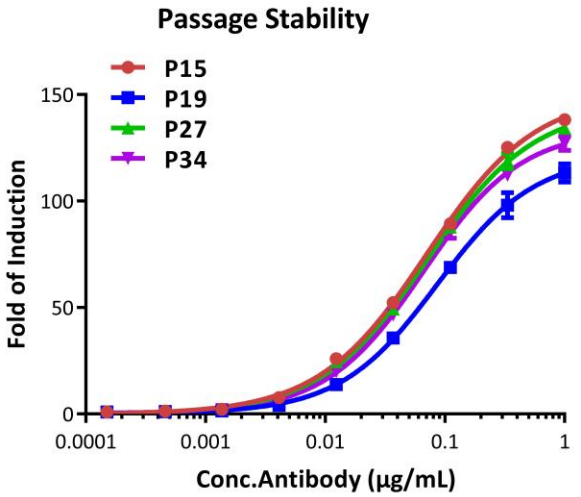
• ***Passage Stability***



	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 (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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• *Related Products*

Products	Cat.No.
Human CD16a (158V) (Luc) Jurkat Reporter Cell	SCJUR-STF067
Human CD16a (158F) (Luc) Jurkat Reporter Cell	SCJUR-STF068
Human CD32a (Luc) Jurkat Reporter Cell	SCJUR-STF069
Human CD32a (131R) (Luc) Jurkat Reporter Cell	SCJUR-STF070
Human CD32b (Luc) Jurkat Reporter Cell	SCJUR-STF071
Human CD64 (Luc) Jurkat Reporter Cell	SCJUR-STF072
Monoclonal Anti-Human CD3 Antibody	CDE-M120a