

Human CD64 (Luc) Jurkat Reporter Cell Data Sheet

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Human CD64 (Luc) Jurkat Reporter Cell Data Sheet

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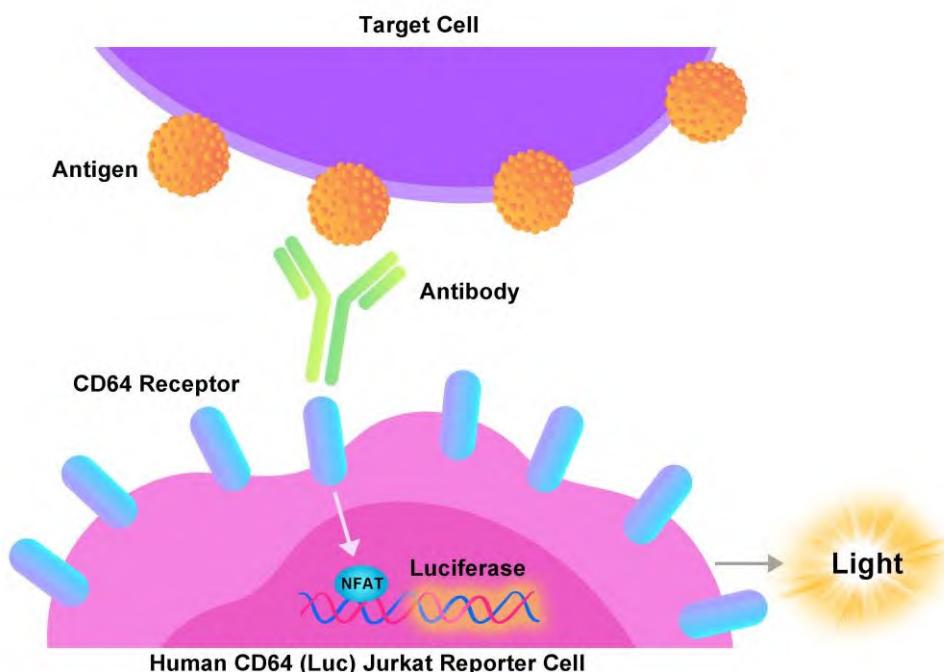
Catalog No.	Size
SCJUR-STF072	2 × (1 vial contains ~5×10 ⁶ cells)

• Description

The Human CD64 (Luc) Jurkat Reporter Cell was engineered to not only express the NFAT response element driving luciferase expressing systems, but also express the receptor full length human CD64 (Uniprot: P12314-1), which can be used to evaluate ADCP activity of antibodies in the presence of corresponding target cells. When co-cultured with a target cell and relevant antibody, the antibody simultaneously binds the target cell antigen and CD64 receptor on the surface of Human CD64 (Luc) Jurkat Reporter Cell, resulting in receptor clustering, intracellular signaling and NFAT-mediated luminescence.

• Application

- Determination of ADCP activity induced by antibodies.



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• Cell Line Profile

Cell line	Human CD64 (Luc) Jurkat Reporter Cell
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
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)
- 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)

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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°C with 5% CO₂ 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

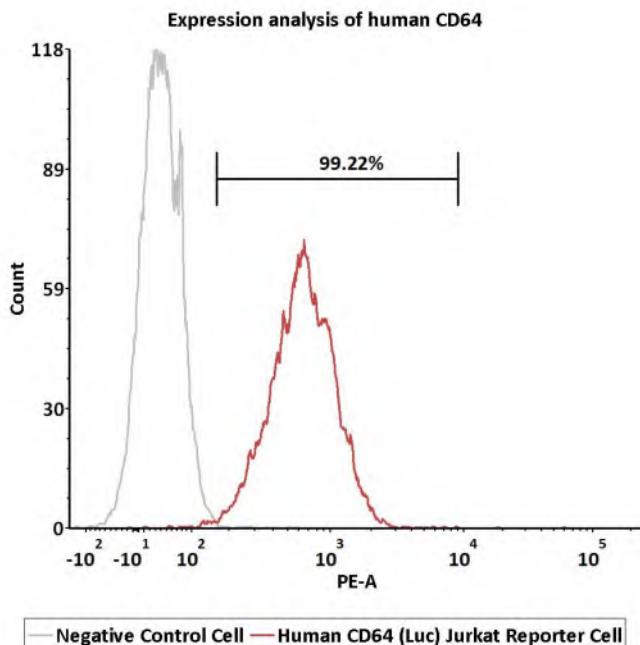


Fig1. Expression analysis of human CD64 on Human CD64 (Luc) Jurkat Reporter Cell by FACS. Human CD64 (Luc) Jurkat Reporter Cell or negative control cell were stained with PE-labeled anti-human CD64 antibody.

• Application

Determination of ADCP activity (RLU)

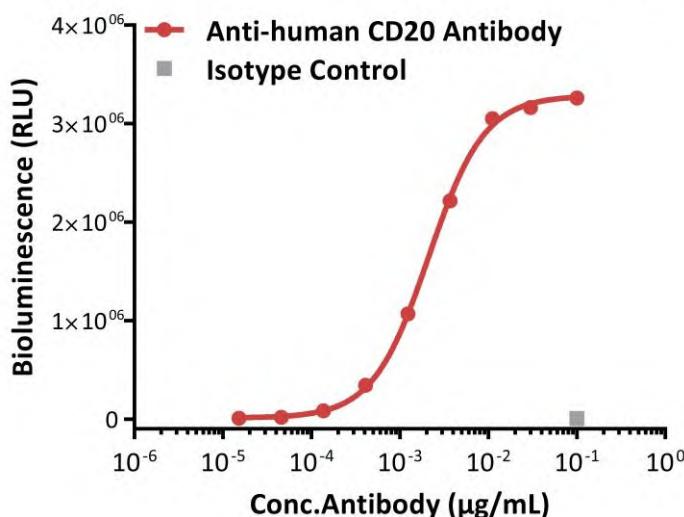


Fig2. ADCP response to anti-human CD20 antibody (RLU). Anti-human CD20 antibody-induced ADCP activity was evaluated using Human CD64 (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The EC50 of anti-human CD20 antibody was approximately 0.0021 $\mu\text{g/mL}$.

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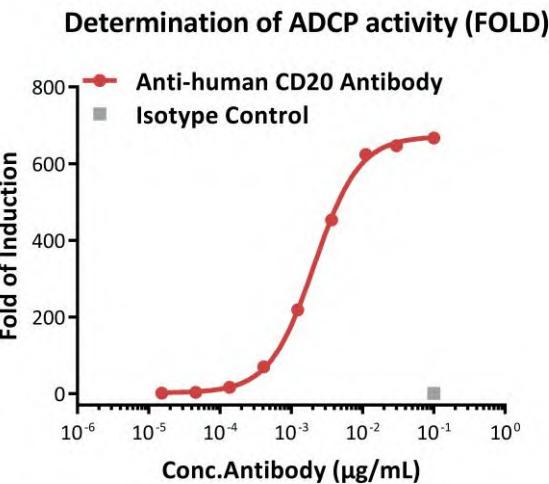
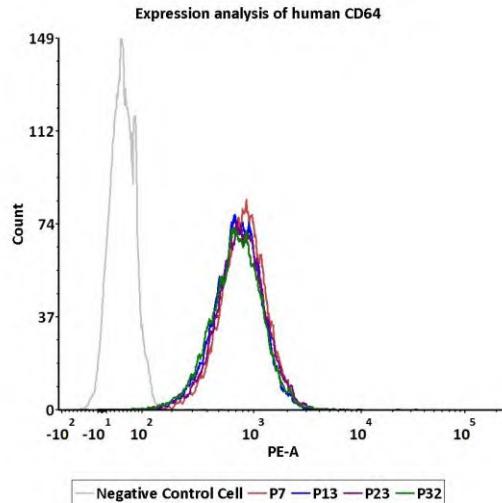


Fig3. ADCP response to anti-human CD20 antibody (FOLD). Anti-human CD20 antibody-induced ADCP activity was evaluated using Human CD64 (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The max induction fold was approximately 667.

• Passage Stability



Passage	MFI for CD64 (PE)
P7	772
P13	699
P23	727
P32	678

Fig4. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human CD64 on Human CD64 (Luc) Jurkat Reporter Cell demonstrates consistent mean fluorescent intensity across passage 7-32.

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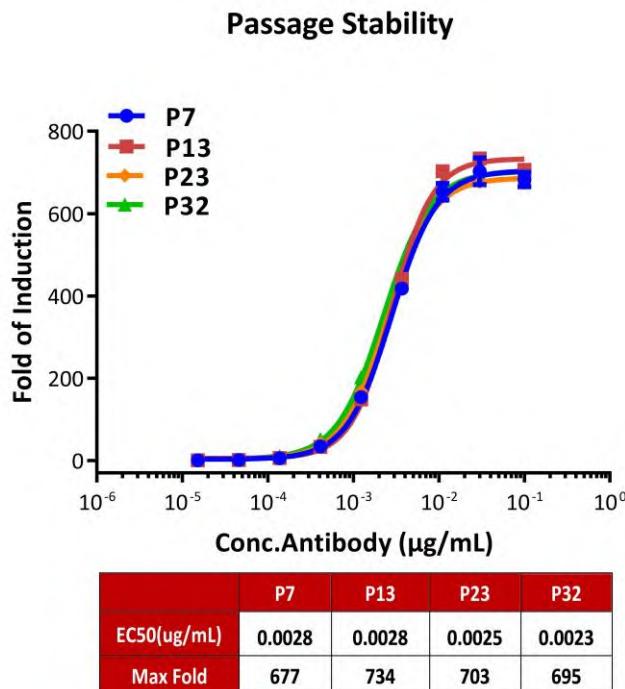


Fig5. Passage stability analysis by Signaling Bioassay. The continuously growing Human CD64 (Luc) Jurkat Reporter Cell was stimulated with serial dilutions of anti-human CD20 antibody in the presence of Raji cells that express CD20 endogenously. Anti-human CD20 antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 7-32.

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• *Related Products*

<u>Products</u>	<u>Cat.No.</u>
Human CD16a (158V) (Luc) Jurkat Reporter Cell	SCJUR-STF067
Human CD16a (158F) (Luc) Jurkat Reporter Cell	SCJUR-STF068
Human CD32a (131H) (Luc) Jurkat Reporter Cell	SCJUR-STF069
Human CD32a (131R) (Luc) Jurkat Reporter Cell	SCJUR-STF070
Human CD32b (Luc) Jurkat Reporter Cell	SCJUR-STF071