

Human CD32a (131R) (Luc) Jurkat Reporter Cell Data Sheet

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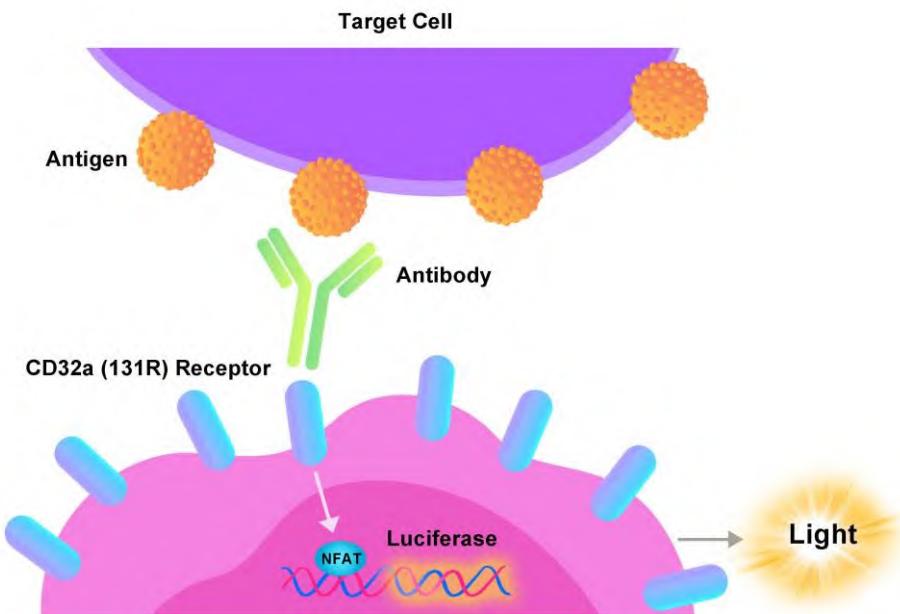
| Catalog No. | Size |
|--------------|--|
| SCJUR-STF070 | 2 × (1 vial contains ~5×10 ⁶ cells) |

• Description

The Human CD32a (131R) (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 CD32a (Uniprot: P12318-1) mutated to an Arginine (R) at amino acid 131 exhibiting a lower affinity for IgG2 isotypes compared to CD32a-131H, 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 CD32a (131R) receptor on the surface of Human CD32a (131R) (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 CD32a (131R) (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**

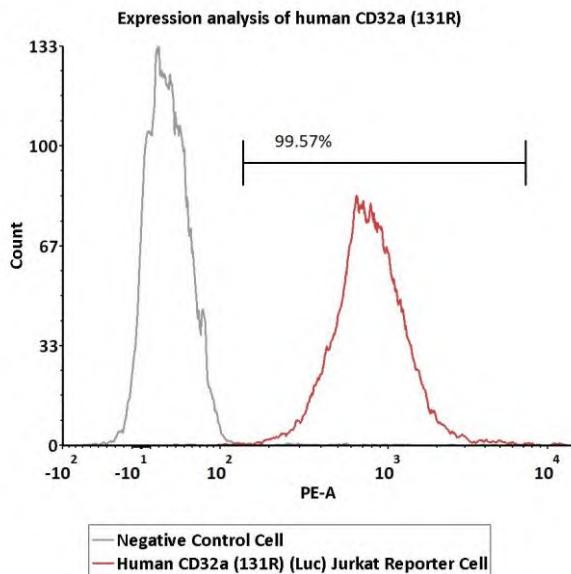


Fig1. Expression analysis of human CD32a (131R) on Human CD32a (131R) (Luc) Jurkat Reporter Cell by FACS. Cell surface staining was performed on Human CD32a (131R) (Luc) Jurkat Reporter Cell or negative control cell using PE-labeled anti-human CD32a antibody.

- **Application**

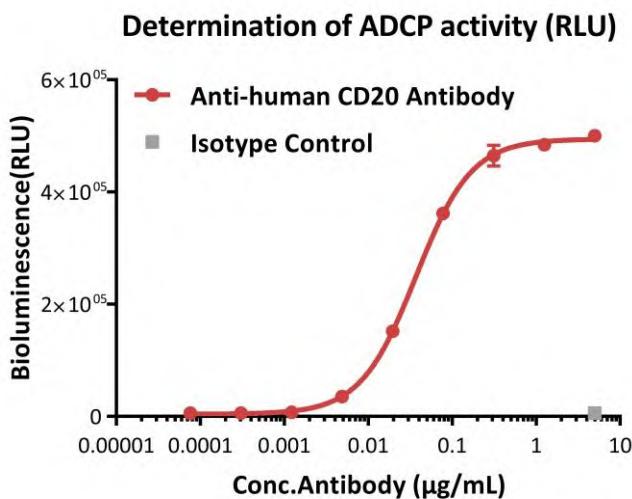


Fig2. ADCP response to anti-human CD20 antibody (RLU). Anti-human CD20 antibody-induced ADCP activity was evaluated using Human CD32a (131R) (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The EC50 of anti-human CD20 antibody was approximately 0.037 μ g/mL.

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Determination of ADCP activity (FOLD)

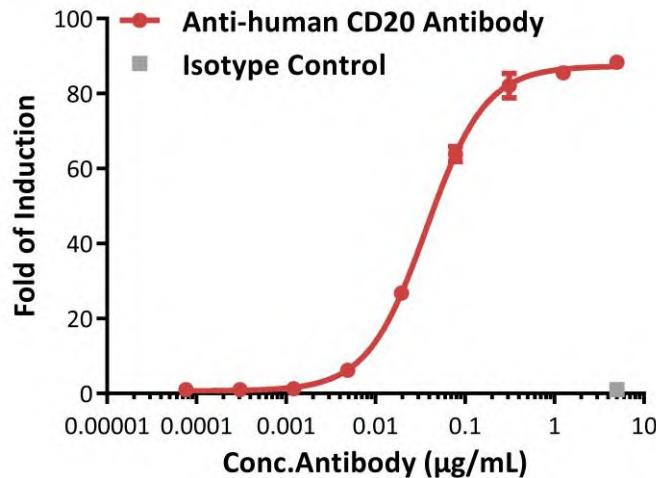


Fig3. ADCP response to anti-human CD20 antibody (FOLD). Anti-human CD20 antibody-induced ADCP activity was evaluated using Human CD32a (131R) (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The max induction fold was approximately 88.

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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 CD32b (Luc) Jurkat Reporter Cell | SCJUR-STF071 |
| Human CD64 (Luc) Jurkat Reporter Cell | SCJUR-STF072 |