

Human CD16a (158F) (Luc) Jurkat Reporter Cell Development Service Data Sheet

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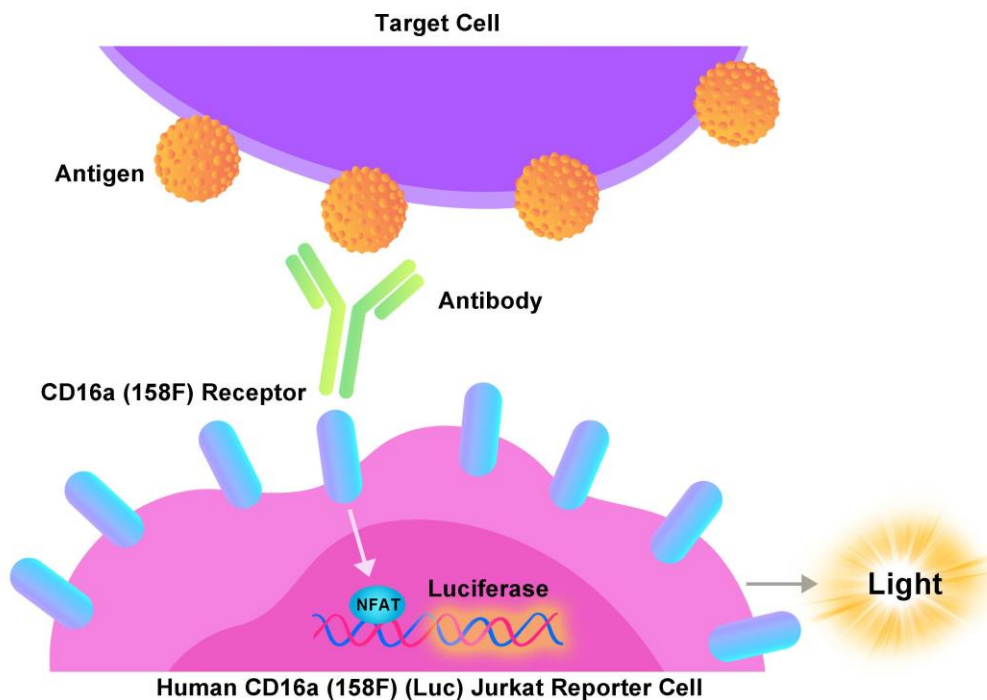
Catalog No.	Size
SCJUR-STF068	2 × (1 vial contains ~5×10 ⁶ cells)

• *Description*

The Human CD16a (158F) (Luc) Jurkat Reporter Cell was engineered to not only express the NFAT response element driving luciferase expressing systems, but also express full length human CD16a (158F) receptor (Gene ID: 2214) exhibiting a lower affinity for IgG1 and IgG3 isotypes compared to CD16a-158V, which can use to evaluate ADCC 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 CD16a (158F) receptor on the surface of Human CD16a (Luc) (158F) Jurkat Reporter Cell, resulting in receptor clustering, intracellular signaling and NFAT-mediated luminescence.

• *Application*

- Determination of ADCC activity induced by antibodies.



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• Cell Line Profile

Cell line	Human CD16a (158F) (Luc) Jurkat Reporter Cell
Host Cell	Jurkat
Property	Suspension
Complete Growth Medium	RPMI-1640 + 10% FBS
Selection Marker	Puromycin (5 µg/mL) + Hygromycin (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)
- Complete Growth Medium: RPMI-1640 + 10% FBS
- Culture Medium: RPMI-1640 + 10% FBS, Hygromycin (20 µg/mL), 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)
- CO₂ 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°C with 5% CO₂ 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*

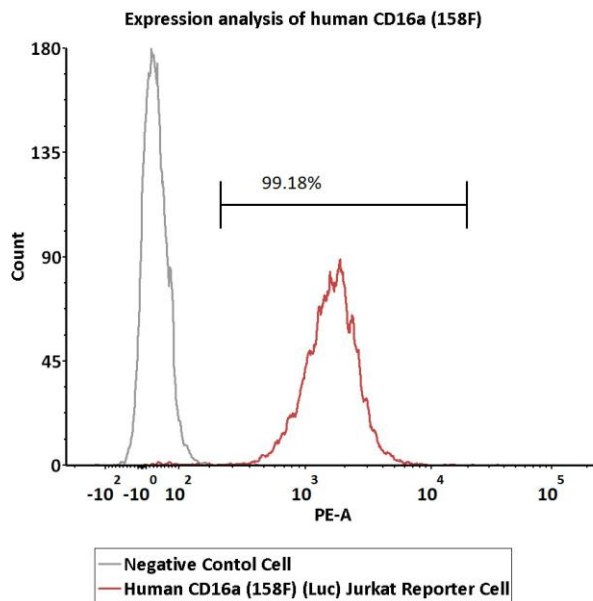


Fig1. Expression analysis of human CD16a (158F) on Human CD16a (158F) (Luc) Jurkat Reporter Cell by FACS. Human CD16a (158F) (Luc) Jurkat Reporter Cell or negative control cell were stained with PE-labeled Anti-Human CD16a antibody.

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• *Application*

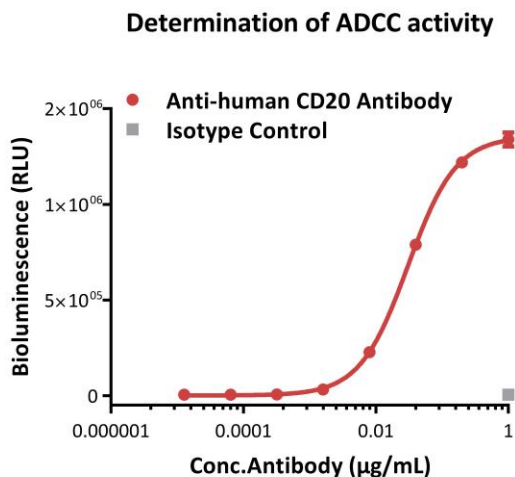


Fig2. ADCC response to anti-human CD20 antibody (RLU). Anti-human CD20 antibody-induced ADCC activity was evaluated using Human CD16a (158F) (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The EC50 of anti-human CD20 antibody was approximately 0.03 µg/mL.

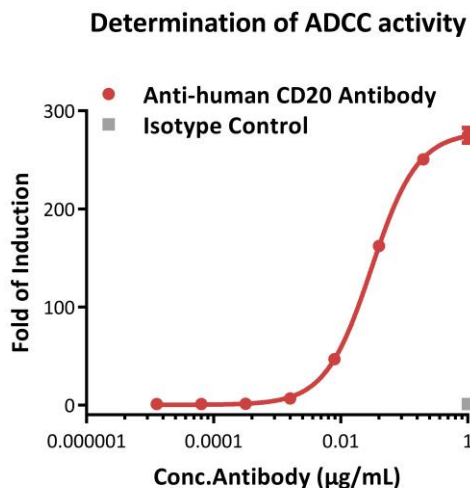
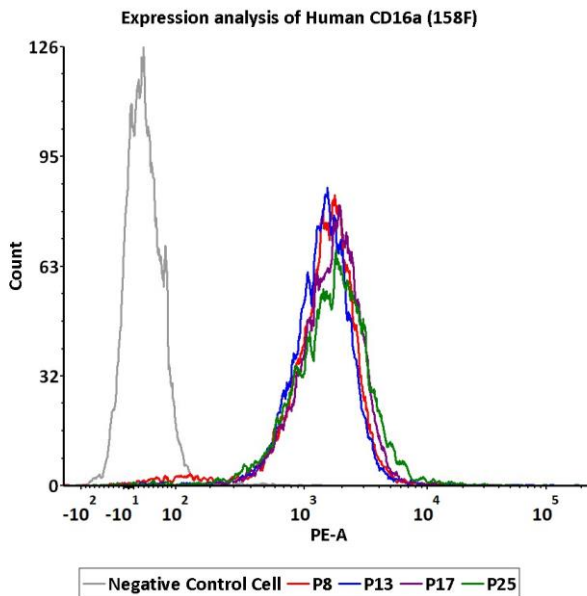


Fig3. ADCC response to anti-human CD20 antibody (Fold). Anti-human CD20 antibody-induced ADCC activity was evaluated using Human CD16a (158F) (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The max induction fold was approximately 275.

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• Passage Stability



Passage	MFI for CD16a (158F) (PE)
P8	1467.63
P13	1384.55
P17	1635.15
P25	1652.56

Fig4. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human CD16a (158F) on Human CD16a (158F) (Luc) Jurkat Reporter Cell demonstrates consistent mean fluorescent intensity across passage 8-25.

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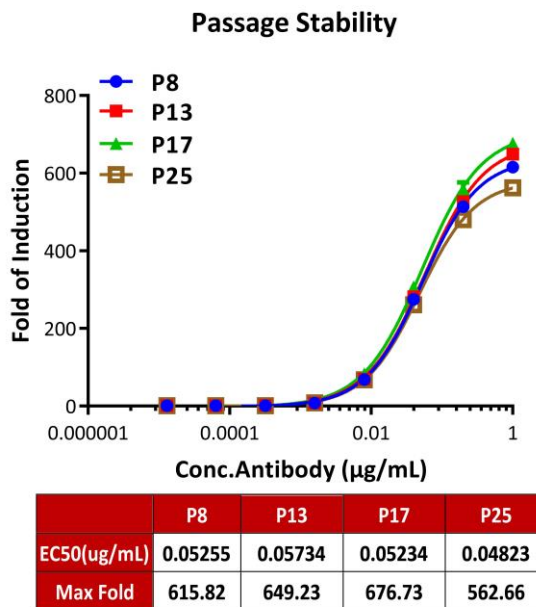


Fig5. Passage stability analysis by Signaling Bioassay. The continuously growing Human CD16a (158F) (Luc) Jurkat Reporter Cell was stimulated with serial dilutions of Anti-human CD20 antibody in the presence of Raji cells. Anti-human CD20 antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 8-25.

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• *License Disclosure*

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• *Related Products*

<u>Products</u>	<u>Cat.No.</u>
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