Human CD32a (131H) (Luc) Jurkat Reporter Cell

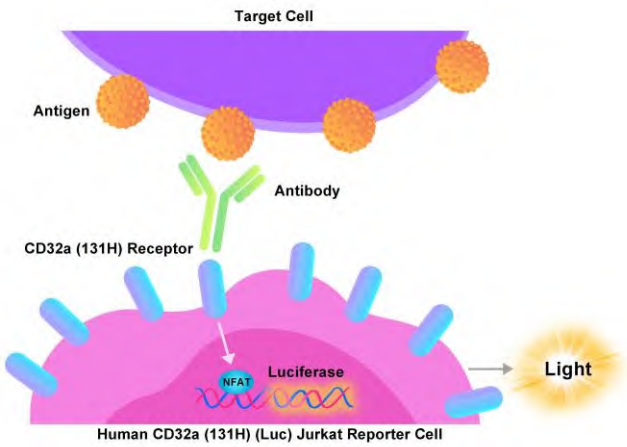
Catalog No.	Size
SCJUR-STF069	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human CD32a (131H) (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 (131H) (Gene ID: 2212) exhibiting a higher affinity for IgG2 isotypes compared to CD32a-131R, which can use 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 (131H) receptor on the surface of Human CD32a (131H) Jurkat Reporter Cell, resulting in receptor clustering, intracellular signaling and NFAT-mediated luminescence.

Application

- Determination of ADCP activity induced by antibodies.



• *Cell Line Profile*

Cell line	Human CD32a (131H) (Luc) Jurkat Reporter Cell
Host Cell	Jurkat
Property	Suspension
Complete Growth Medium	RPMI-1640 + 10% FBS
Selection Marker	Hygromycin (20 μg/mL) + Puromycin (5 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	16-20 hours
Transduction Technique	Lentivirus

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• *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)
- CO_2 Incubator (Thermo, 3111)
- Biological Safety Cabinet (Thermo, 1389)

• *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% CO_2 incubator.

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• *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*

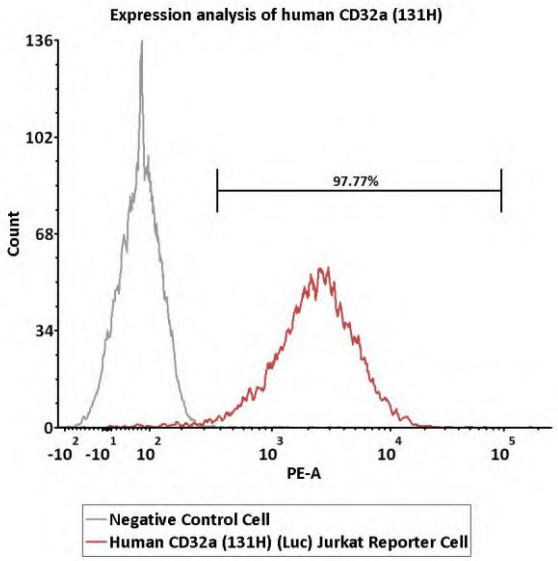


Fig1. Expression analysis of human CD32a on Human CD32a (131H) (Luc) Jurkat Reporter Cell by FACS. Human CD32a (131H) (Luc) Jurkat Reporter Cell or negative control cell were stained with PE-labeled anti-Human CD32a (131H) antibody.

• *Application*

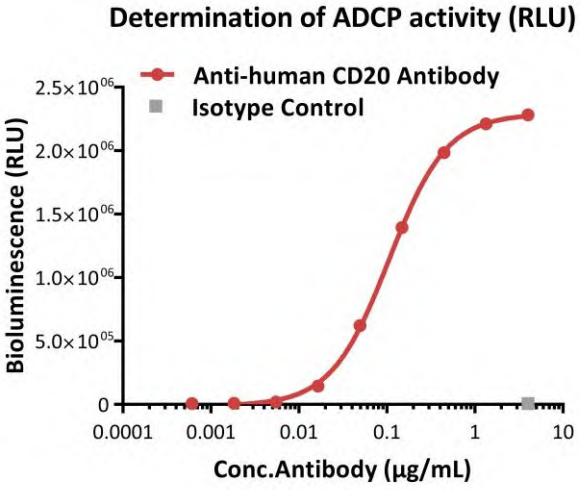


Fig2. ADCP response to anti-human CD20 antibody (RLU). Anti-human CD20 antibody-induced ADCP activity was evaluated using Human CD32a (131H) (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The EC50 of anti-human CD20 antibody was approximately 0.1054 μg/mL.

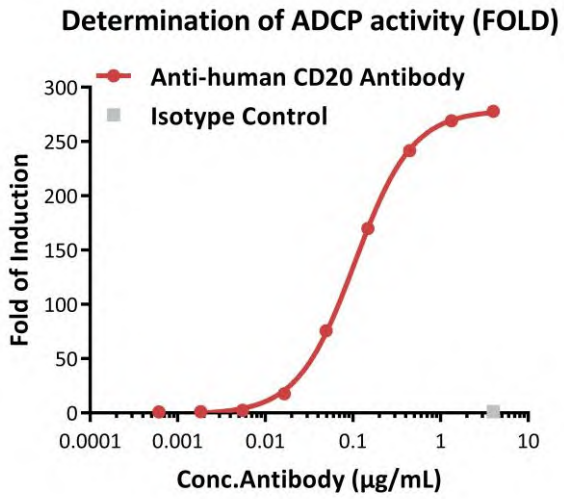
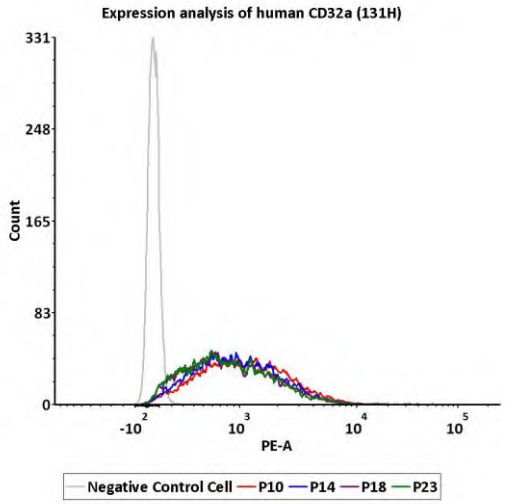


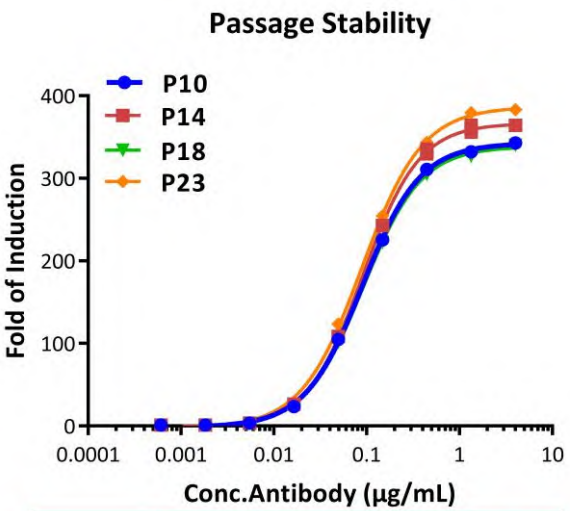
Fig3. ADCP response to anti-human CD20 antibody (FOLD). Anti-human CD20 antibody-induced ADCP activity was evaluated using Human CD32a (131H) (Luc) Jurkat Reporter Cell in the presence of Raji cells that express CD20 endogenously. The max induction fold was approximately 277.90.

- ***Passage Stability***



Passage	MFI for CD32a (131H) (PE)
P10	989
P14	859
P18	725
P23	725

Fig4. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human CD32a (131H) on Human CD32a (131H) (Luc) Jurkat Reporter Cell demonstrates consistent mean fluorescent intensity across across passage 10-23.



	P10	P14	P18	P23
EC50 (μg/mL)	0.09047	0.09133	0.09199	0.08891
Max Fold	342.78	364.11	340.08	383.04

Fig5. Passage stability analysis by anti-human CD32 antibody stimulation. The continuously growing Human CD32a (131H) (Luc) Jurkat Reporter Cell was stimulated with serial dilutions of antibodies. Anti-human CD20 antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 10-23.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Human CD16a (158V) (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF067
Human CD16a (158F) (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF068
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