Human PD-1/LAG-3 (Luc) Jurkat Reporter Cell Development Service Data Sheet

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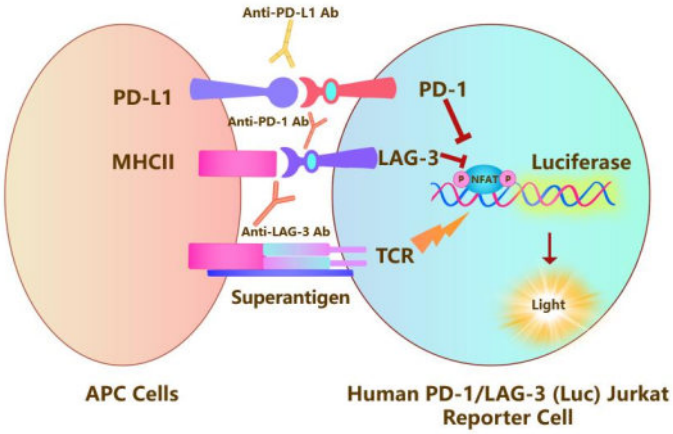
Catalog No.	Size
SCJUR-STF063	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The Human PD-1/LAG-3 (Luc) Jurkat Reporter Cell was engineered to not only express the NFAT response element driving luciferase expressing systems, but also express the receptors full length human PD-1 (Gene ID: 5133) and LAG-3 (Gene ID: 3902), which can use to evaluate the synergistic effect of anti-human PD-1 and anti-human LAG-3 antibody. When co-cultured with target cells expressing human PD-L1 and MHCII, the PD-1/PD-L1 and LAG-3/MHCII interactions inhibit TCR signaling and NFAT-mediated luminescence. Blocking the PD-1/PD-L1 and LAG-3/MHCII interactions by the stimultaneous addition of anti-PD-1 or anti-PD-L1 and anti-LAG-3 antibodies release the inhibitory signals and result in TCR activation and NFAT-mediated luminescence.

• *Application*

- Screen for anti-human PD-1 or/and anti-human LAG-3 antibody.



• *Cell Line Profile*

Cell line	Human PD-1/LAG-3 (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℃ with 5% CO_2 incubator.

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• *Subculture*

Adjust the cell density at 2 x10^5-5 x10^5 viable cells/mL by the addition of fresh culture medium or replacement of culture medium. Do not allow the cell density to exceed 3 x10^6 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*

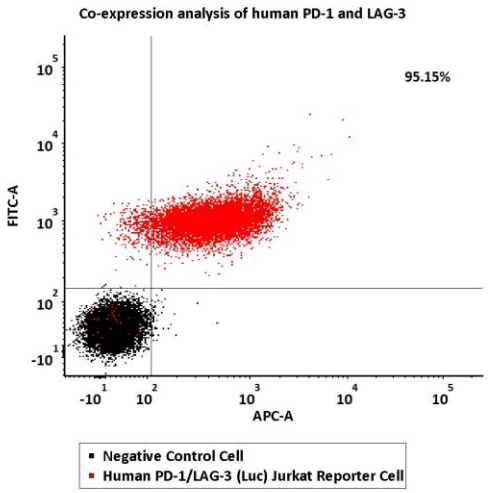


Fig1. Co-expression analysis of human PD-1 and LAG-3 on Human PD-1/LAG-3 (Luc) Jurkat Reporter Cell by FACS. Cell surface staining was performed on Human PD-1/LAG-3 (Luc) Jurkat Reporter Cell or negative control cell using FITC-labeled anti-human PD-1 antibody and APC-labeled anti-human LAG-3 antibody.

• *Application*

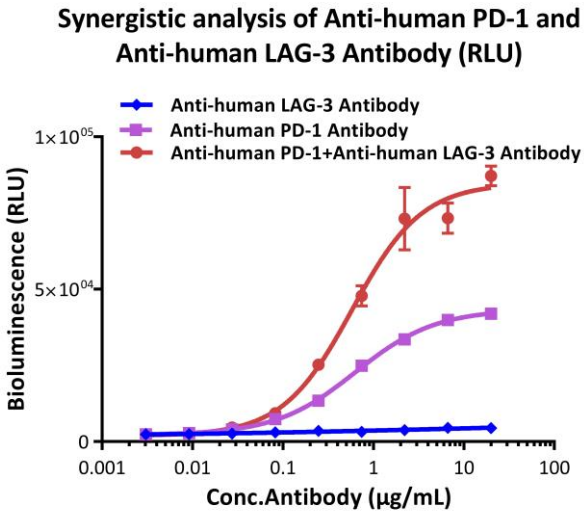


Fig2. Analysis of the synergistic effect for anti-human PD-1 and anti-human LAG-3 antibody（RLU）. This reporter cell was co-incubated with serial dilutions of anti-human PD-1 plus anti-human LAG-3 antibody in the presence of target cells expressing human PD-L1 and MHCII. The EC50 was approximately 0.58 μg/mL.

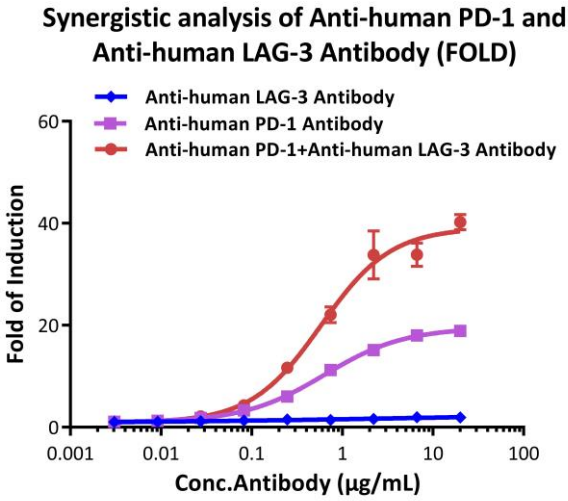
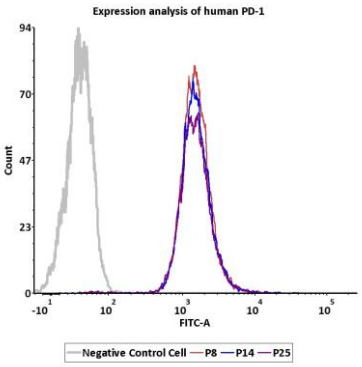


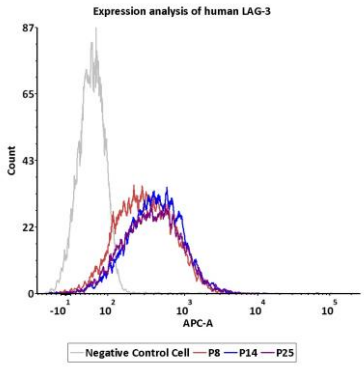
Fig3. Analysis of the synergistic effect for anti-human PD-1 and anti-human LAG-3 antibody（FOLD）. This reporter cell was co-incubated with serial dilutions of anti-human PD-1 plus anti-human LAG-3 antibody in the presence of target cells expressing human PD-L1 and MHCII. The max induction fold was approximately

- ***Passage Stability***

A



B



Passage	MFI for PD-1 (FITC)	MFI for LAG-3 (APC)
P8	1374.00	298.00
P14	1391.00	396.00
P25	1391.00	367.00

Fig4. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human PD-1 and LAG-3 on Human PD-1/LAG-3 (Luc) Jurkat Reporter Cell demonstrates consistent mean fluorescent intensity across passage 8-25. (A) Human PD-1 expression analysis. (B) Human LAG-3 expression analysis.

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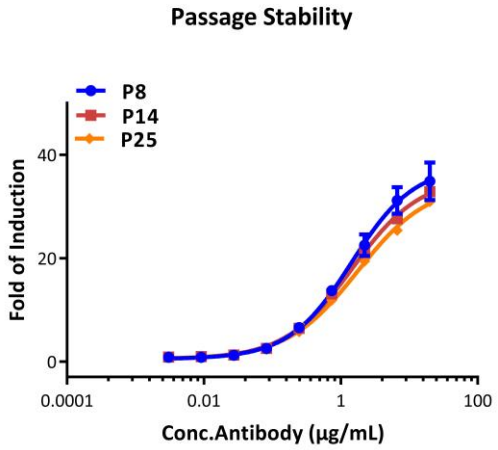

	P8	P14	P25
EC50(ug/mL)	1.44	1.49	1.77
Max Fold	34.87	32.85	30.93

Fig5. Passage stability analysis by Signaling Bioassay. The continuously growing Human PD-1/LAG-3 Jurkat Reporter Cell was stimulated with serial dilutions of anti-human PD-1 plus anti-human LAG-3 antibody in the presence of target cells expressing PD-L1 and MHCII. Anti-human PD-1 plus anti-human LAG-3 antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 8-25.

• *License Disclosure*

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• *Related Products*

Products	Cat.No.
Human PD-1 (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF064
Human LAG-3 (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF065
Human TIGIT (Luc) Jurkat Reporter Cell Development Service	SCJUR-STF066