

Human PD-1 (Luc) Jurkat Reporter Cell

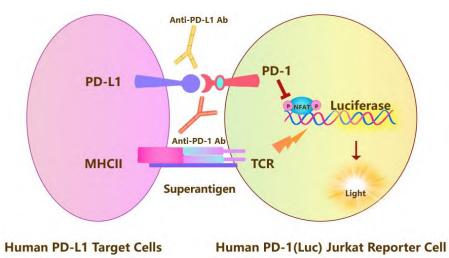
Catalog No.	Size
SCJUR-STF064	$2 \times (1 \text{ vial contains } \sim 5 \times 10^{6} \text{ cells})$

• Description

The Human PD-1 (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 PD-1 (Gene ID: 5133), which can use to evaluate the potency of PD-1 blockade. When cocultured with target cells expressing human PD-L1, the PD-1/PD-L1 interaction inhibits TCR signaling and NFAT-mediated luminescence. Blocking the PD-1/PD-L1 interaction by either anti-PD-1 or anti-PD-L1 antibodies releases the inhibitory signal and results in TCR activation and NFAT-mediated luminescence.

• Application

• Screen for anti-human PD-1 or anti-human PD-L1 antibody.





Cell line	Human PD-1 (Luc) Jurkat Reporter Cell	
Host Cell	Jurkat	
Property	Suspension	
Complete Growth Medium	RPMI-1640 + 10% FBS	
Selection Marker	Hygromycin (20 μ g/mL) + Puromycin (5 μ g/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, 1% P/S
- Culture Medium: RPMI-1640 + 10% FBS, Hygromycin (20 µg/mL), Puromycin (5 µg/mL), 1%P/S
- 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)

• 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



• Receptor Assay

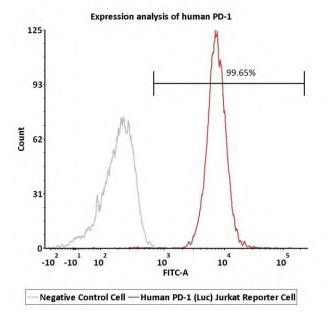


Fig1. Expression analysis of human PD-1 on Human PD-1 (Luc) Jurkat Reporter Cell by FACS. Cell surface staining was performed on Human PD-1 (Luc) Jurkat Reporter Cell or negative control cell using FITC-labeled anti-human PD-1 antibody.

• Application

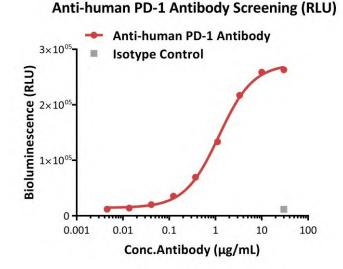


Fig2. Blocking activity of anti-human PD-1 antibody (RLU). This reporter cell was incubated with serial dilutions of Anti-human PD-1 Antibody in the presence of target cells expressing human PD-L1. The EC50was approximately 1.189 μg/mL.





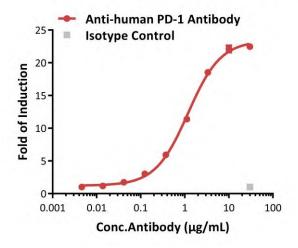
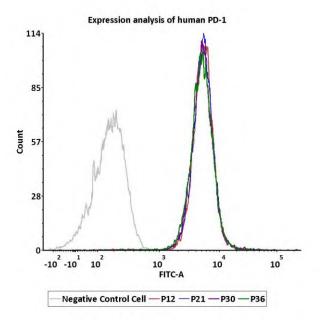


Fig3. Blocking activity of anti-human PD-1 antibody (FOLD). This reporter cell was incubated with serial dilutions of Anti-human PD-1 Antibody in the presence of target cells expressing human PD-L1. The max induction fold was approximately 22.47.



• Passage Stability



Passage	MFI for PD-1 (FITC)
P12	5184
P21	5137
P30	4998
P36	4922

Fig4. Passage stability analysis of receptors expression by FACS. Flow cytometry surface staining of human PD-1 on Human PD-1 (Luc) Jurkat Reporter Cell demonstrates consistent mean fluorescent intensity across passage 12-36.



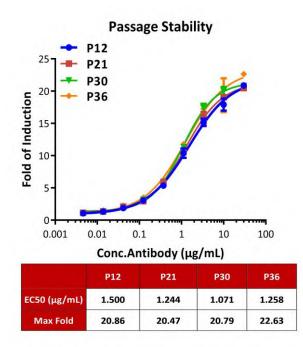


Fig5. Passage stability analysis by Signaling Bioassay. The continuously growing Human PD-1 (Luc) Jurkat Reporter Cell was stimulated with serial dilutions of Anti-human PD-1 antibody in the presence of target cells expressing human PD-L1. Anti-human PD-1 antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 12-36.

• License Disclosure

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• Related Products

Products	<u>Cat.No.</u>
Human LAG-3 (Luc) Jurkat Reporter Cell	SCJUR-STF065
Human TIGIT (Luc) Jurkat Reporter Cell	SCJUR-STF066