Human PD-1 (Luc) Jurkat Reporter Cell Data Sheet

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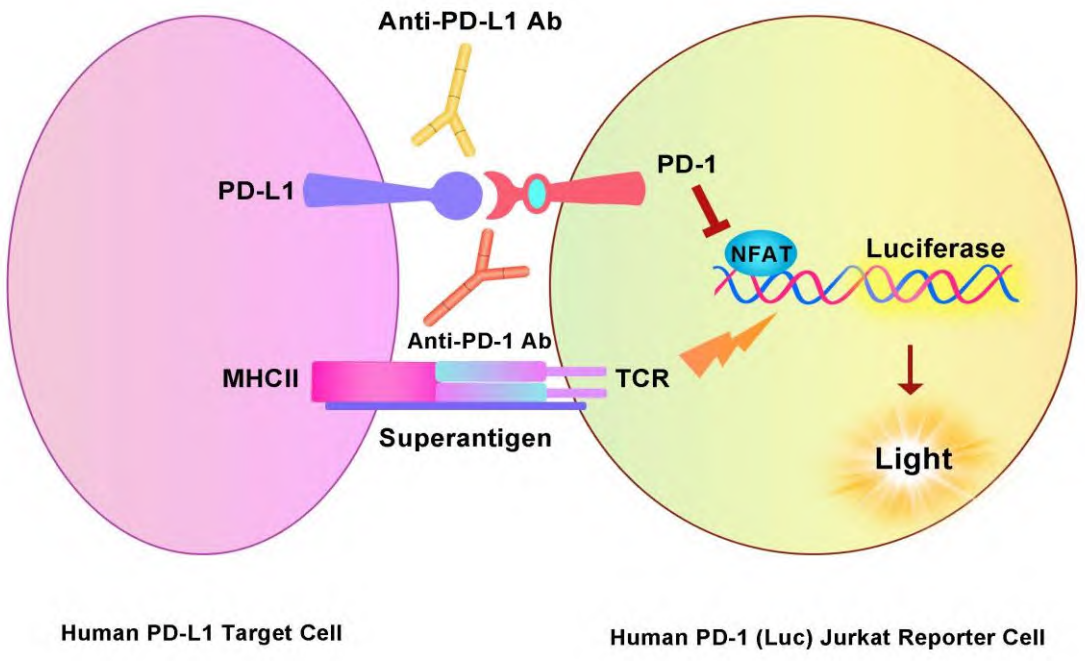
Catalog No.	Size
SCJUR-STF064	2 × (1 vial contains ~5×10^6 cells)

• *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 (Uniprot: Q15116), 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 antagonistic antibody or anti-human PD-L1 antibody.
- Screen for anti-human PD-1 agonistic antibody.



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• *Cell Line Profile*

Cell line	Human PD-1 (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℃ with 5% CO_2 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.

• *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***

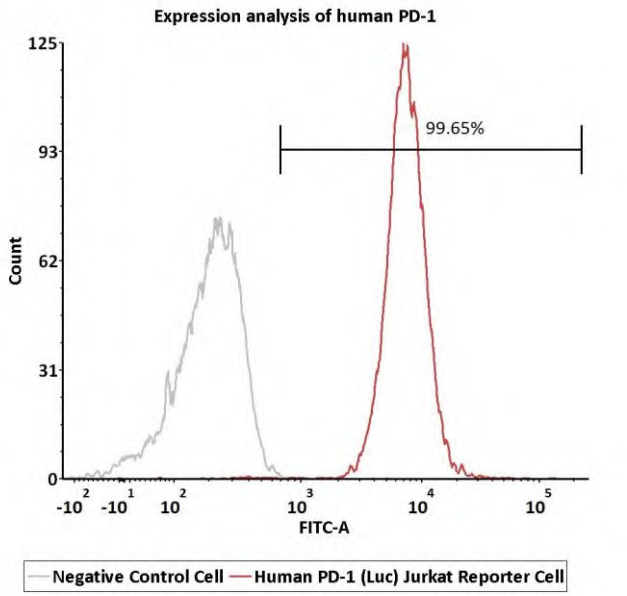

Fig1. Expression analysis of human PD-1 on Human PD-1 (Luc) Jurkat Reporter Cell by FACS. Cell surface staining was performed on Human PD-1 (Luc) Jurkat Reporter Cell or negative control cell using FITC-labeled anti-human PD-1 antibody.

- ***Application***

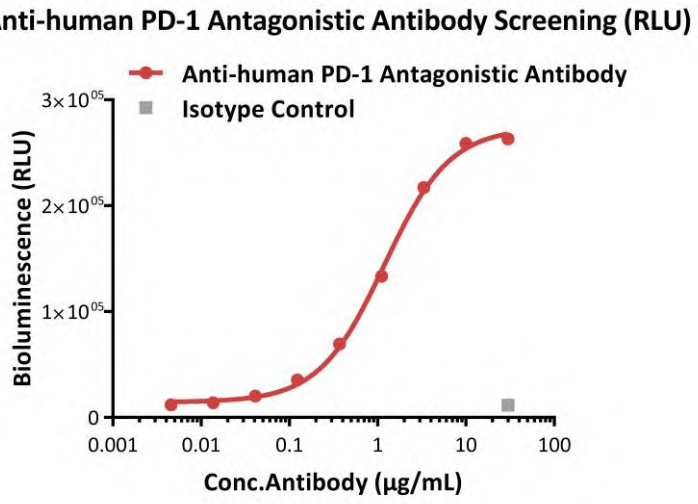

Fig2. Blocking activity of anti-human PD-1 antagonistic antibody (RLU). This reporter cell was incubated with serial dilutions of anti-human PD-1 antagonistic antibody in the presence of target cells expressing human PD-L1. The EC50 was approximately 1.189 μg/mL.

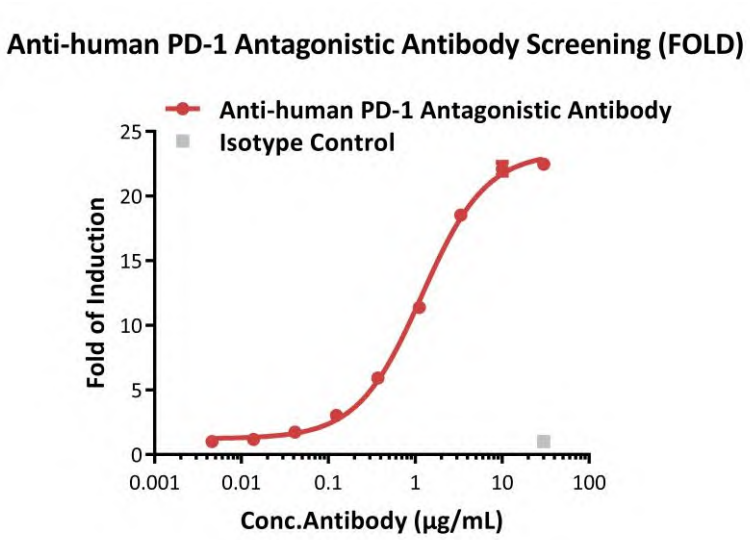

Fig3. Blocking activity of anti-human PD-1 antagonistic antibody (FOLD). This reporter cell was incubated with serial dilutions of anti-human PD-1 antagonistic antibody in the presence of target cells expressing human PD-L1. The max induction fold was approximately 22.47.

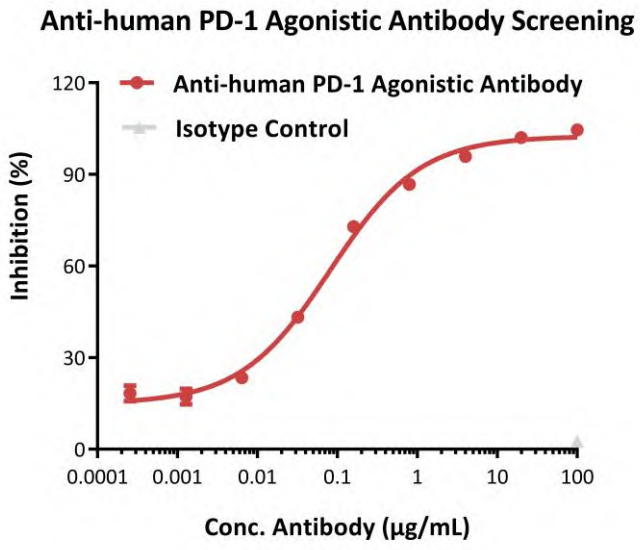

Fig4. Agonistic activity analysis of anti-human PD-1 agonistic antibody. This reporter cell was incubated with serial dilutions of anti-human PD-1 agonistic antibody (Rosnilimab). The EC50 of anti-human PD-1 agonistic antibody (Rosnilimab) is approximately 0.08197 μg/mL.

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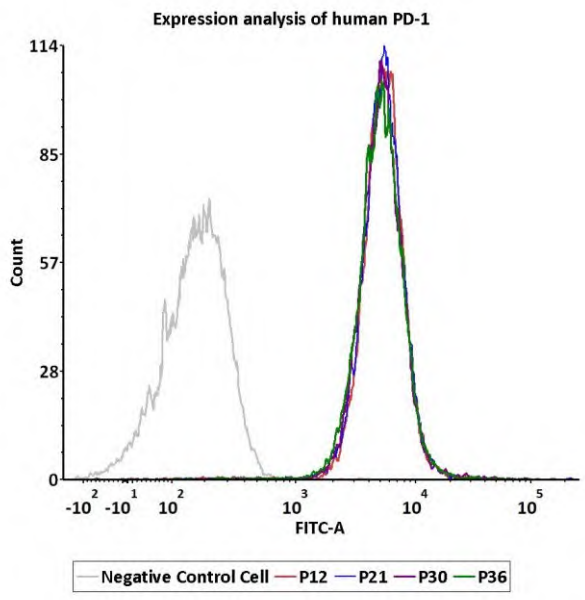
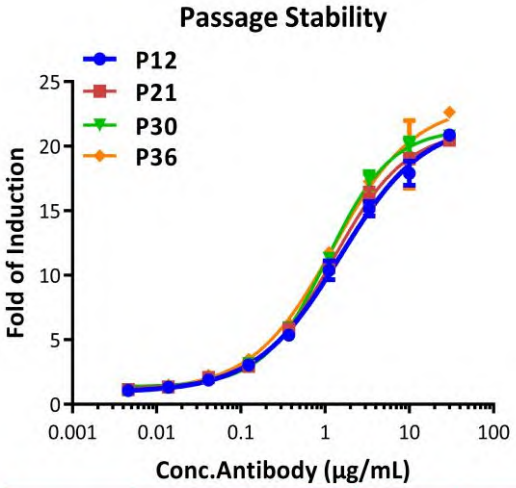
- ***Passage Stability***



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

Fig5. Passage stability analysis of receptor 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.



	P12	P21	P30	P36
EC50 (μg/mL)	1.500	1.244	1.071	1.258
Max Fold	20.86	20.47	20.79	22.63

Fig6. 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 antagonistic antibody in the presence of target cells expressing human PD-L1. Anti-human PD-1 antagonistic antibody stimulated response demonstrates passage stabilization (fold induction and EC50) across passage 12-36.

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• *Related Products*

Products	Cat.No.
HEK293/Human PD-L1, GFP Tag Stable Cell Line	CHEK-ATP002
HEK293/Human 4-1BB Ligand / TNFSF9 Stable Cell Line	CHEK-ATP039
HEK293/Human 4-1BB / TNFRSF9 Stable Cell Line	CHEK-ATP038
Human PD-1/LAG-3 (Luc) Jurkat Reporter	SCJUR-STF063
Human LAG-3 (Luc) Jurkat Reporter Cell	SCJUR-STF065
Raji/Human PD-L1 Stable Cell Line	SCRAJ-STT075
Raji/Human CD155 Stable Cell Line	SCRAJ-STT076
CHO/Human LILRB4 Stable Cell Line	SCCHO-ATP087
HEK293/Human LILRB4 Stable Cell Line	CHEK-ATP088
Raji/Human HVEM Stable Cell Line	SCRAJ-STF108
CHO/Human LIGHT Stable Cell Line	SCCHO-ATP109
CHO/Human BTLA Stable Cell Line	SCCHO-ATP110
HEK293/Human PD-1 Stable Cell Line	CHEK-ATP143
HEK293/Human HVEM Stable Cell Line	CHEK-ATP147
HEK293/Human NKp46 Stable Cell Line	CHEK-ATP153
HEK293/Human ITPRIPL1 Stable Cell Line	CHEK-ATP203
Human NKp46 (Luc) Jurkat Reporter Cell	SCJUR-STF130