CHO/Human PD-L1 Stable Cell Line (Low Expression) Data Sheet

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CHO/Human PD-L1 Stable Cell Line (Low Expression)

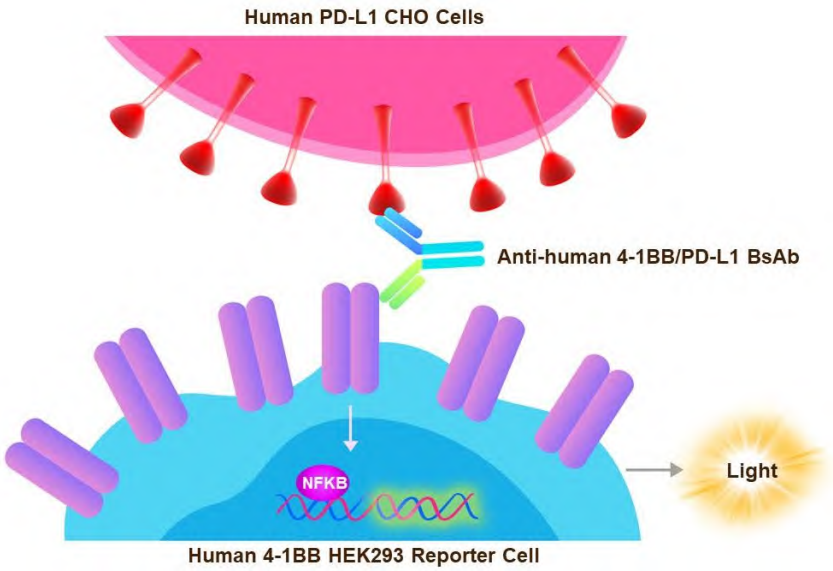
Catalog No.	Size
SCCHO-ATP077L	2 × (1 vial contains ~5×10^6 cells)

• *Description*

The CHO/Human PD-L1 Stable Cell Line was engineered to express the receptor full length human PD-L1 (Uniprot: Q9NZQ7-1), with different levels of PD-L1 expression (High, Medium, Low), which can be used to mimic cancer target cells with various PD-L1 expression levels. When co-cultured with Human 4-1BB HEK293 Reporter Cell and anti-human 4-1BB/PD-L1 BsAb, the anti-human 4-1BB/PD-L1 BsAb can be crosslinked, thereby strengthening 4-1BB pathway-activated luminescence.

• *Application*

- Useful for cell-based PD-L1 binding assay
- Useful for PD-L1-mediated crosslinking



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• *Cell Line Profile*

Cell line	CHO/Human PD-L1 Stable Cell Line (Low Expression)
Host Cell	CHO
Property	Adherent
Complete Growth Medium	F-12K + 10% FBS
Selection Marker	Puromycin (2 μg/mL)
Incubation	37°C with 5% CO_2
Doubling Time	22-24 hours
Transduction Technique	Lentivirus

• *Materials Required for Cell Culture*

- F-12K Nutrient Mixture (Gibco, Cat. No. 21127-022)
- Fetal bovine serum (CellMax, Cat. No. SA211.02)
- Puromycin (InvivoGen, Cat. No. ant-pr-5b)

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.

- 0.25% Trypsin-EDTA (1X), Phenol Red (Gibco, Cat. No. 25200-056)
- Penicillin-Streptomycin (Gibco, Cat. No. 15140-122)
- Phosphate Buffered Saline (1X) (HyClone, Cat. No. SH30256.01)
- Complete Growth Medium: F-12K + 10% FBS, 1%P/S
- Culture Medium: F-12K + 10% FBS, Puromycin (2 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)
- CO_2 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. Centrifuge at approximately 1000 rpm for 5 minutes.
4. Resuspend the cell pellet with 5 mL complete growth medium and transfer the cell suspension into a T-75 flask containing 10-15 mL of pre-warmed complete growth medium.
5. Incubate at 37°C with 5% CO_2 incubator until the cells are ready to be split.

• *Subculture*

1. Cell viability may be low after thawing, and full recovery may take up to a week. Monitor the cells daily until the culture reaches 80-90% confluency. At this point, remove and discard the spent medium. Avoid allowing the cells to become over-confluent to ensure optimal cell health.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 3 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 5-7 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of culture medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps.
5. Transfer appropriate aliquots of the cell suspension to a new T-75 flask. A subcultivation ratio of 1:6 to 1:10 is recommended. Adjust the ratio based on your specific culture system.
6. Incubate at 37°C with 5% CO_2 incubator.
7. When the cell culture reaches 80-90% confluency, proceed to the next subculture. Avoid over-confluency, as this may negatively impact cell performance in subsequent passages.

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, transition to the culture medium containing the selection marker during subculturing.

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• *Cryopreservation*

1. When the cell culture reaches 80-90% confluency, remove and discard the spent medium.
2. Wash the cells once with sterile PBS. Avoid adding PBS directly onto the cell surface.
3. Add 3 mL of 0.25% Trypsin-EDTA to the T-75 flask. Place the flask at 37°C for 5-7 minutes, until 90% of the cells have detached. Monitor under a microscope to avoid over-trypsinization.
4. Add 6.0 to 8.0 mL of complete growth medium using a pipette and gently rinse the cells from the surface of the T-75 flask. Gently pipette up and down several times to achieve a single cell suspension without cell clumps. Count the viable cells.
5. Transfer the cell suspension to a centrifuge tube. Centrifuge at 1000 rpm for 5 min at room temperature to pellet the cells.
6. After centrifugation, discard the supernatant. Resuspend the cells in ice cold freezing medium to a concentration of 5×10^6 to 1×10^7 cells/mL.
7. 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 Condition*

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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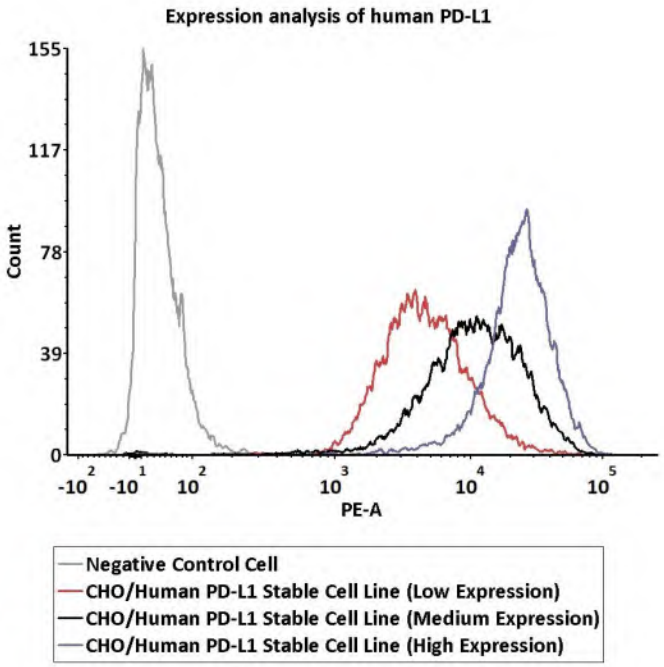
- ***Receptor Assay***



Catalog No.	Stable Cell Line	MFI for PD-L1 (PE)
SCCHO-ATP077L	CHO/Human PD-L1 Stable Cell Line (Low Expression)	4193.33
SCCHO-ATP077M	CHO/Human PD-L1 Stable Cell Line (Medium Expression)	10543.80
SCCHO-ATP077H	CHO/Human PD-L1 Stable Cell Line (High Expression)	22783.64

Fig1. Expression analysis of human PD-L1 on CHO/ Human PD-L1 Stable Cell Line by FACS. Cell surface staining using PE-labeled anti-human PD-L1 antibody was performed on CHO/Human PD-L1 Stable Cell Line with different expression levels: CHO/Human PD-L1 Stable Cell Line (Low Expression); CHO/Human PD-L1 Stable Cell Line (Medium Expression); CHO/Human PD-L1 Stable Cell Line (High Expression).

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• *Related Products*

Products	Cat.No.
CHO/Human CD16a (158V) Stable Cell Line (Low Expression)	SCCHO-ATP059L
CHO/Human CD16a (158V) Stable Cell Line (Medium Expression)	SCCHO-ATP059M
CHO/Human CD16a (158V) Stable Cell Line (High Expression)	SCCHO-ATP059H
CHO/Human CD32b Stable Cell Line (Low Expression)	SCCHO-ATP060L
CHO/Human CD32b Stable Cell Line (Medium Expression)	SCCHO-ATP060M
CHO/Human CD32b Stable Cell Line (High Expression)	SCCHO-ATP060H
CHO/Human CD32a Stable Cell Line (Low Expression)	SCCHO-ATP061L
CHO/Human CD32a Stable Cell Line (Medium Expression)	SCCHO-ATP061M
CHO/Human CD32a Stable Cell Line (High Expression)	SCCHO-ATP061H
CHO/Human CD64 Stable Cell Line (Low Expression)	SCCHO-ATP062L
CHO/Human CD64 Stable Cell Line (Medium Expression)	SCCHO-ATP062M
CHO/Human CD64 Stable Cell Line (High Expression)	SCCHO-ATP062H
CHO/Human PD-L1 Stable Cell Line (High Expression)	SCCHO-ATP077H
CHO/Human PD-L1 Stable Cell Line (Medium Expression)	SCCHO-ATP077M