293T Cyno PD-L1 Cell Line

Cat. No: KC-1001 Version 18102501

I. Cell Line Information

Catalog number	KC-1001
Cell line name:	293T Cyno PDL1 Cell Line
Gene ID/Accession #:	XM_005581779.2
Host cell line	293T
Cell type:	Human embryonic kidney
Description:	HEK293T cell line stable expressing exogenous cynomolgus PDL1 gene
Quantity:	One vial of frozen cells ($5X10^6$ per vial)
Stability:	Stable in culture over a minimum of 10 passages
Application:	Drug screening and biological assays
Freeze medium:	70% DMEM + 20% FBS + 10% DMSO
Propagation medium:	DMEM + 10% FBS + 0.5ug/ml Puromycin
Selection marker:	Puromycin
Morphology:	Fibroblastoid cells growing as monolayer
Subculture:	Split saturated culture 1:4~1:5 every 2~3 days; seed out at about 1-3 x 10^5 cells/ml
Incubation:	37 °C with 5% CO_2
Doubling time:	Approximately 30 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

II. Background

PD-L1, also called human programed cell death ligand 1, is a transmembrane protein that play a major role in suppressing the immune system during particular events such as pregnancy, tissue allografts, autoimmune disease, virus infection and cancer. PD-L1 binds to its receptor PD-1 on activated T cells, B cells, and myeloid cells, to modulate activation or inhibition. Upregulation of PD-L1 can allow cancer cell to evade the host immune system.

III. Cell Line Generation

293T cyno PD-L1 Cell Line was generated using lentivirus expressing cynomolgus PD-1 gene sequence.

IV. Characterization using FACS

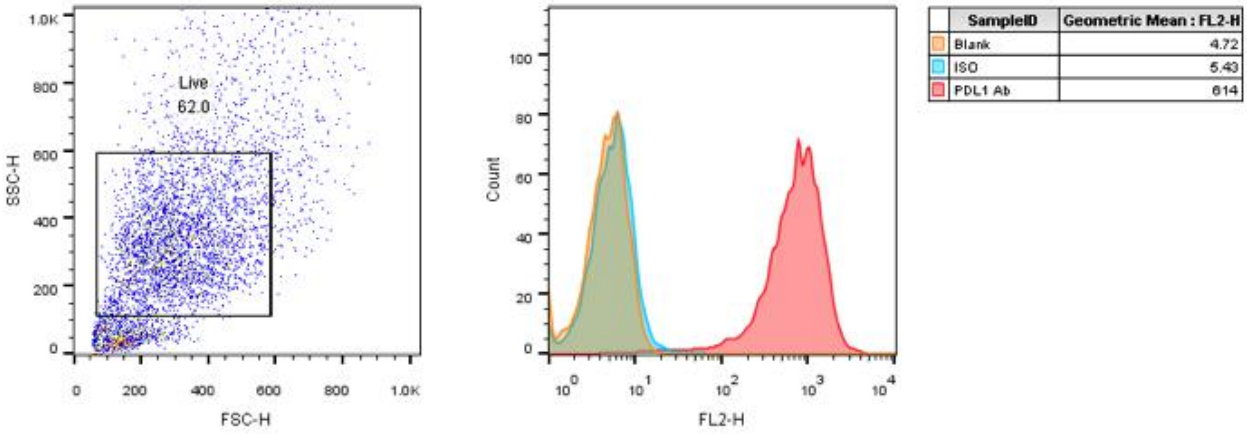

Figure: Characterization of PDL1 overexpressing in 293T stable clones using FACS.

V. Application

A. Hybridoma or binders of PD1/PD-L1 screening with FACS

B. blockage of PD1/PD-L1 binding with FACS

VI. Cell Resuscitation

1. Prewarm culture medium (DMEM supplemented with 10% FBS and 0.5ug/ml puromycin) in a 37°C water bath.
2. Thaw the frozen vial in a 37°C water bath for 1-2 minutes.
3. Transfer the vial into biosafety cabinet, and wipe the surface with 70% ethanol.
4. Unscrew the top of the vial and transfer the cell suspension gently into a sterile centrifuge tube containing 9.0 mL complete culture medium.
5. Spin at ~ 125 x g for 5~7 minutes at room temperature, and discard the supernatant without disturbing the pellet.
6. Resuspend cell pellet with the appropriate volume of complete medium and transfer the cell suspension into a T25 culture flask.
7. Incubate the flask at 37°C, 5% CO_2 incubator.
8. Split saturated culture 1:4 ~ 1:5 every 2~3 days; seed out at about 1-3 x 10^5 cells/ml.

VII. Cell Freezing

1. Prepare the freezing medium (70% DMEM + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.

3. Transfer cells to a sterile, conical centrifuge tube, and count the cells.
4. Centrifuge the cells at 250 x g for 5 minutes at room temperature and carefully aspirate off the medium.
5. Resuspend the cells at a density of at least 3 x10^6 cells/ml in chilled freezing medium.
6. Aliquot 1 ml of the cell suspension into each cryovial.
7. Freeze cells in the CoolCell freezing container overnight in a −80°C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

VIII. References

1. Sunshine, J. & Taube, J. M. PD-1/PD-L1 inhibitors. Current Opinion in Pharmacology 23, 32–38 (2015).
2. Boussiotis, V. A. Molecular and Biochemical Aspects of the PD-1 Checkpoint Pathway. N Engl J Med 375, 1767–1778 (2016).
3. Topalian, S. L. et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med 366, 2443–2454 (2012).

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