

## 293T human PD-1 Cell Line

Cat. No: KC-0204

Version 17103102

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### I. Cell Line Information

<b>Catalog number</b>	KC-0204
<b>Cell line name:</b>	293T human PD1 Cell Line
<b>Gene ID/Accession #:</b>	NM_005018
<b>Host cell line</b>	293T
<b>Cell type:</b>	Human embryonic kidney
<b>Description:</b>	HEK293T cell line stable expressing exogenous human PD1 gene
<b>Quantity:</b>	One vial of frozen cells (5X10 <sup>6</sup> per vial)
<b>Stability:</b>	Stable in culture over a minimum of 10 passages
<b>Application:</b>	Drug screening and biological assays
<b>Freeze medium:</b>	70% DMEM + 20% FBS + 10% DMSO
<b>Propagation medium:</b>	DMEM + 10% FBS + 0.5ug/ml Puromycin
<b>Selection marker:</b>	Puromycin
<b>Morphology:</b>	Fibroblastoid cells growing as monolayer
<b>Subculture:</b>	Split saturated culture 1:4~1:5 every 2~3 days; seed out at about 1-3 x 10 <sup>5</sup> cells/ml
<b>Incubation:</b>	37 °C with 5% CO <sub>2</sub>
<b>Doubling time:</b>	Approximately 30 hours
<b>Mycoplasma status:</b>	Negative
<b>Biosafety level:</b>	1
<b>Storage:</b>	Liquid nitrogen immediately upon receiving

### II. Background

PD-1, human programmed cell death 1 protein, is a receptor belonging to immunoglobulin superfamily which is expressed primarily on activated T cells, NK cells, B cells, and monocytes. PD-1 interaction with its ligands, PD-L1 and PD-L2, leads to downregulation of T cell responses, including T cell proliferation and cytokine production, and limits immune-destruction of tissues. The interaction between PD-1 and its ligands, thus plays a role in

maintaining the balance between immune activation and tolerance, potentially including tumor tolerance.

### III. Cell Line Generation

293T Human PD-1 Cell Line was generated using plasmid Vector with Human PD-1 gene sequence.

### IV. Characterization using FACS

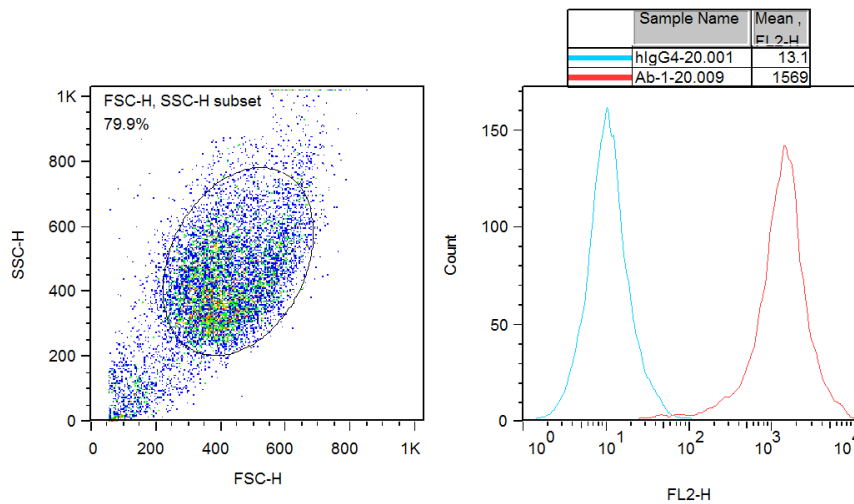


Figure: Characterization of PD1 overexpressing in 293T stable clones using FACS.

### 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<sub>2</sub> incubator.
8. Split saturated culture 1:4 ~ 1:5 every 2~3 days; seed out at about 1-3 x 10<sup>5</sup> 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  $3 \times 10^6$  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  $-80^{\circ}\text{C}$  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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