Human Recombinant PD-1 Stable Cell Line

Cat. No.: M00529 Version 09/05/2018

I Introductioin

Cell Line Name: CHO-K1/PD-1

Gene Synonyms: PDCD1 ; CD279; PD-1; SLEB2; hPD-1; hPD-l; hSLE1

Expressed Gene: Codon Optimized from NM_005018.2; no expressed tags

Host Cell: CHO-K1

Quantity: Two vials of frozen cells (>1×10^6 cells/vial)

Stability: 15 passages

Application: Binding assay or use as immunogen

Freeze Medium: 95% Culture Medium, 5% (V/V) DMSO

Complete Growth Medium: F12K, 10% FBS

Culture Medium: F12K, 10% FBS, 8 μg/ml Puromycin

Mycoplasma Status: Not detected.*

Storage: Liquid nitrogen immediately upon receipt

II Background

Programmed cell death protein 1, also known as PD-1 and CD279 (cluster of differentiation 279), is a protein that in humans is encoded by the PDCD1 gene. PD-1, functioning as an immune check-point, plays an important role in down regulating the immune system by preventing the activation of T-cells, which in turn reduces autoimmunity and promotes self-tolerance. The inhibitory effect of PD-1 is accomplished through a dual mechanism of promoting apoptosis (programmed cell death) in antigen specific T-cells in lymph nodes while simultaneously reducing apoptosis in regulatory T cells (suppressor T cells).

*The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

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III Representative Data

Protein Expression Validation

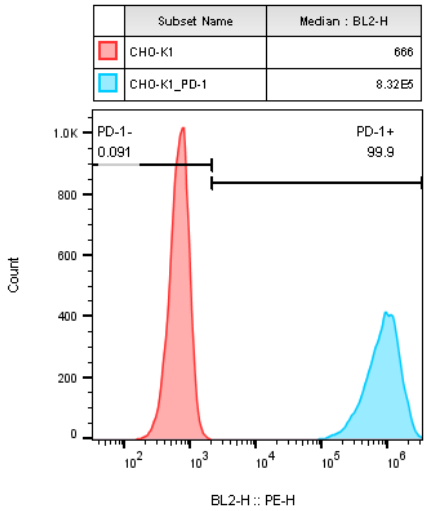

Figure 1: FACS analysis of PD-1 expression in CHO-K1/PD-1 cells.

IV Thawing And Subculturing

1 Thawing Protocol

1. Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
2. Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
3. Pellet cells by centrifugation at 800 rpm for 4 min, and remove the medium.
4. Resuspend the cells in complete growth medium.
5. Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
6. Grow the cells in incubator with 37°C, 5% CO_2.
7. Add antibiotic the following day.

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2 Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.25% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25200-072) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes). Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.
4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 800 rpm for 4 min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5% CO_2.

Subcultivation Ratio: 1:4 to 1:8 weekly.
Medium Renewal: Every 2 to 3 days

V References

1. Mahoney KM1, Rennert PD2, Freeman GJ3. Combination cancer immunotherapy and new immunomodulatory targets. Nat Rev Drug Discov. 2015 Jul 31;14(8):561-84.
2. Francisco LM, Sage PT, Sharpe AH (Jul 2010). "The PD-1 pathway in tolerance and autoimmunity".Immunological Reviews 236: 219–42.

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