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Data Sheet

PD-1 - HEK293 Recombinant Cell Line

Cat #: 60680

Product Description
Recombinant HEK293 stably expressing human PD-1 (Programmed Cell Death 1, PDCD1, SLEB2, CD279, GenBank Accession #NM_005018).

Background
The binding of Programmed Cell Death Protein 1 (PD-1), a receptor expressed on activated T cells, to its ligands, PD-L1 and PD-L2, negatively regulates immune responses. The PD-1 ligands are found on most cancers, and PD-1:PD-L1/2 interaction inhibits T cell activity and allows cancer cells to escape immune surveillance. The PD-1:PD-L1/2 pathway is also involved in regulating autoimmune responses, making these proteins promising therapeutic targets for a number of cancers, as well as multiple sclerosis, arthritis, lupus, and type I diabetes.

Application
- Suitable for screening for PD-1-binding antibodies screening and biologial assays in a cellular context.

Format
Each vial contains ~2 X 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing
The cell line has been screened using the metabolite-based Mycoplasma Detection Kit (Biotool, #B3903) to confirm the absence of Mycoplasma species.

General Culture Conditions

Thaw Medium 1 (BPS Cat. #60187): MEM medium (Hyclone, #SH30024.01) + 10% FBS (Life Technologies, #26140-079) + 1% non-essential amino acids (Hyclone, #SH30238.01) + 1 mM Na pyruvate (Hyclone, #SH30239.01) + 1% Penicillin/Streptomycin (Hyclone, SV30010.01)

Growth Medium 1F (BPS Cat. #79540) : Thaw Medium 1 (BPS Bioscience, #60187) plus 100 μg/ml of Hygromycin B (Life Technologies, #10687-010)

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Cells should be grown at 37°C with 5% CO_2 using Growth Medium 1F to ensure recombinant expression. PD-1 HEK293 cells should display a typical cell division time of about 24 hours.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 (**no Hygromycin B)**, spin down cells at 1000 rpm, and resuspend cells in 5 ml of pre-warmed Thaw Medium 1 (**no Hygromycin B)**. Transfer resuspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 (**no Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch, to Growth Medium 1F (**contains Hygromycin B)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from the culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 1F (**contains Hygromycin B)** and transfer to a tube. Spin down cells, resuspend cells in Growth Medium 1F (**contains Hygromycin B)** and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:5 to 1:10 weekly or twice a week.

Note: Just after thawing and at low density, the cells may grow at a slower rate. It is recommended to split the cells with ~ 1:4 ratio at the beginning of culturing. After several passages, the cell growth rate increases and the cells can be split at a higher ratio.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 1 (**no Hygromycin B)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) to ~$2x10^6$ cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage.
It is recommended to expand the cells and freeze down more than 10 vials of cells at an early passage for future use.

Validation
Cell surface expression of human PD-1 in PD-1-HEK293 cells was confirmed by FACS.

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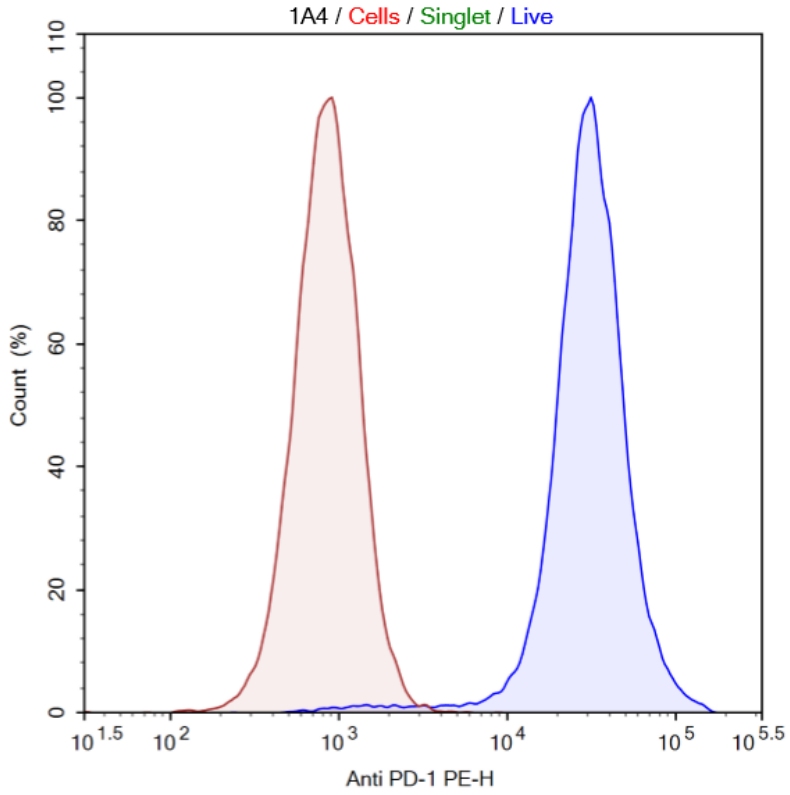
Figure 1. FACS analysis of cell surface expression of PD-1 in PD-1-HEK293 cells. PD-1-HEK293 cells (blue) or control HEK293 cells (red) were stained with PE-labeled Anti-PD-1 Antibody (BPS Bioscience, #71290) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.



	Samples	Subset	Cell Count
	PD-1-HEK293 Cell	Live Singlet	16269
	Control HEK293 Cell	Live Singlet	13,008

Sequence

hPD-1 sequence (accession #NM_005018)

MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCSFSNT
SESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSVVRARRNDS
GTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQTLVVGVVGGL

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LGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDYGELDFQWREKTPE
PPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPEDGHCSWPL

Related Products

Product	Cat. #	Size
PD-1/NFAT Reporter-Jurkat cell line	60535	2 vials
TCR Activator/PD-L1-CHO recombinant cell line	60536	2 vials
PD-L1-CHO cell line	60543	2 vials
Anti-PD-1 Antibody, PE-labeled	71290-1	50 µg
Anti-PD-1 Antibody, PE-labeled	71290-2	100 µg
Thaw Medium 1	60187	100 ml

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