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- Gefahrgutzuschlag
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Data Sheet

CTLA4 - HEK293 Recombinant Cell Line

Cat #: 60681

Product Description
Recombinant HEK293 stably expressing human CTLA4 (Cytotoxic T-Lymphocyte Associated Protein; CD152), GenBank Accession #NM_005214.

Background
CTLA4 is a member of the immunoglobulin superfamily. It is expressed by activated T cells and transmits an inhibitory signal to T cells. CTLA4 is homologous to the T-cell co-stimulatory protein, CD28, and both molecules bind to CD80 (B7-1) and CD86 (B7-2) on antigen-presenting cells. CTLA-4 binds CD80 and CD86 with greater affinity and avidity than CD28 thus enabling it to outcompete CD28 for its ligands and act as an "off" switch when bound to CD80 or CD86. Not surprisingly, CTLA-4 is an important drug target for the regulation of the host's response to cancer.

Application
- Screening for CTLA4 binding molecules (such as anti-CTLA4 antibody) in a cellular context

Format
Each vial contains ~3 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 Bioscience, #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 Bioscience #79540): Thaw Medium 1 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. CTLA4-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)**. Transfer resuspended cells to 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) and detach cells from 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 with 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) at ~$2x10^6$ 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 for future use at early passage.

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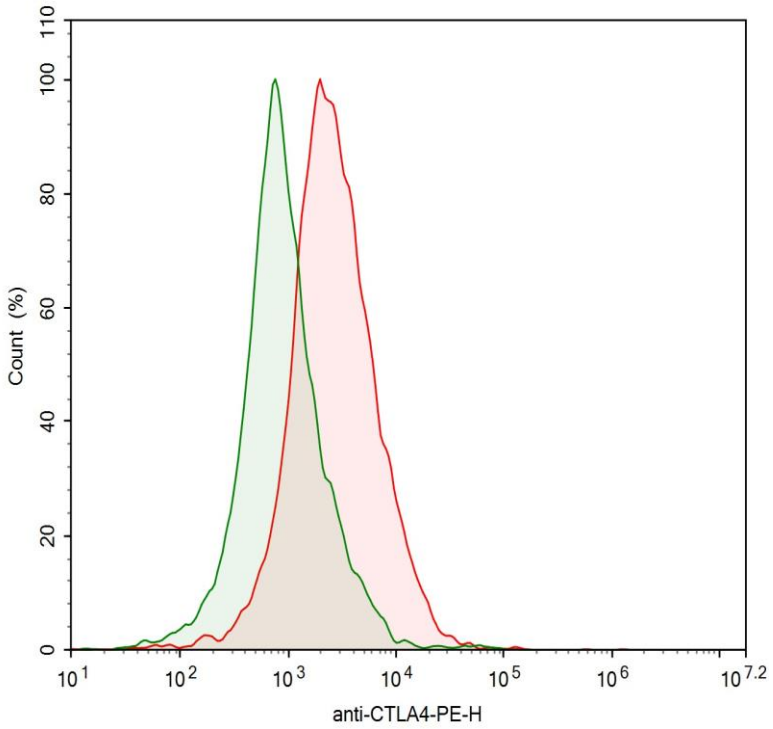
Validation
Cell surface expression of human CTLA4 in CTLA4-HEK293 cells was confirmed by flow cytometry.

Figure 1. Flow cytometry analysis of cell surface expression of CTLA4 in CTLA4-HEK293 cells.
CTLA4-HEK293 cells (red) or control HEK293 cells (green) were stained with PE-labeled Anti-CTLA Antibody (Biolegend, #349906) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.



	Samples	Subset	Cell Count
	Control HEK293 Cell	Live Singlet	3.297
	CTLA4-HEK293 Cell	Live Singlet	8,190

Sequence
CTLA4 sequence (accession number NM_005214)

MACLGFQRHKAQLNLATRTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIASFVCE
YASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQ
GLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDFLLWILAAVSSGLFFY
SFLLTAVSLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPY

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Related Products

Product	Cat. #	Size
Anti-CTLA Neutralizing Antibody	71212	100 µg
NF-kB reporter (Luc) - Jurkat Cell line	60651	2 vials
Thaw Medium 1	60187	100 ml
CTLA4:B7-1 TR-FRET Assay Kit	72120	384 reactions
CTLA4 (CD152), Fc fusion, Biotin-labeled (Human)	71152	50 µg
CTLA4 (Mouse), Fc-Fusion (Human), Avi-Tag	79062	100 ug
CTLA4 (Mouse), Fc-Fusion (Human), Avi-Tag, Biotin	79001	50 µg
B7-1 (CD80), FLAG-Avi-His-Tag	71261	100 µg
B7-1 (CD80), Fc fusion (Human) HiP™	71125	100 µg
B7-1 (CD80), Fc fusion, Biotin-labeled (Human) HiP™	71114	50 µg
B7-2 (CD86), Fc fusion (Human)	71150	100 µg
B7-2 (CD86), Fc fusion, Biotin labeled (Human) HiP™	71159	50 µg
CTLA4:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72009	96 reactions
CTLA4[Biotinylated]:B7-2 Inhibitor Screening Assay Kit	72024	96 reactions
PD-L1:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72026	96 reactions

Notes

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