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Data Sheet

TIGIT - HEK293 Recombinant Cell Line

Cat #: 79332

Product Description

Recombinant HEK293 stably expressing human TIGIT (T cell immunoreceptor with Ig and ITIM domains; VSTM3; VSIG9), GenBank Accession #NM_173799.

Background

TIGIT is a co-inhibitory receptor that is highly expressed in Natural Killer (NK) cells, activated CD4+, CD8+ and regulatory T cells. Interaction with the poliovirus receptor (PVR; CD155) on antigen presenting cells, such as dendritic cells, recruits Src homology (SH) domain-containing protein tyrosine phosphatase SHP1 and SHP2 or the inositol phosphatase SHIP1 and SHIP2 to the TIGIT ITIM domain. This increases IL-10 release and suppresses NF- κ B and NFAT T cell receptor (TCR) signaling, which blocks T cell proliferation and cytokine production. It serves as a competitive inhibitor of CD226, a co-stimulatory receptor for CD155. TIGIT targeting antibodies that block this T cell-intrinsic inhibitory effects have shown enhanced anti-tumor and anti-viral functions in preclinical studies.

Application

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

Format

Each vial contains $\sim 3 \times 10^6$ 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 μ g/ml of Hygromycin B (Life Technologies, #10687-010).

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Cells should be grown at 37°C with 5% CO₂ using Growth Medium 1F to ensure recombinant expression. TIGIT-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% CO₂ incubator overnight. The next day, replace the medium with fresh warm Thaw Medium 1 (**no Hygromycin B**), and continue growing culture in a CO₂ 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 ~2 x 10⁶ 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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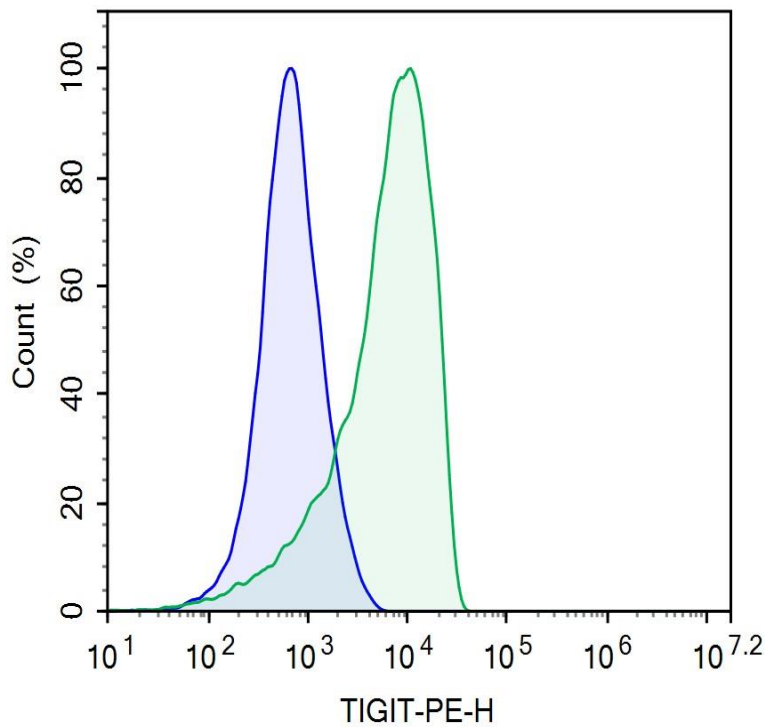
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Validation

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

Figure 1. Flow cytometry analysis of cell surface expression of TIGIT in TIGIT-HEK293 cells.

TIGIT-HEK293 cells (green) or control HEK293 cells (blue) were stained with RPE-labeled Anti-TIGIT Antibody (BPS Bioscience, #71228) 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	32,568
	TIGIT-HEK293 Cell	Live Singlet	36,781

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Sequence

TIGIT sequence (accession number NM_173799)

MACLGFRHKAQLNLRTRWPCTLLFFLLFIPVFCAMHVAQPAVVLASSRGIASFVCE
YASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQVNLTIQ
GLRAMDTGLYICKVELMYPPYYLIGINGTQIYVIDPEPCPDSDFLWILAAVSSGLFFY
SFLLTAVSLSKMLKRSPLTTGVYVKMPTEPECEKQFQPY

Materials Required But Not Provided

- Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1F (BPS Cat. #79540)
- Anti-TIGIT Neutralizing Antibody, PE-Labeled (BPS Cat. #71228)

Related Products

<u>Product</u>	<u>Cat. #</u>	<u>Size</u>
Anti-TIGIT Neutralizing Antibody	71340	100 µg
TIGIT/NFAT Reporter (Luc) - Jurkat Cell line	60538	2 vials
Thaw Medium 1	60187	100 ml
Anti-TIGIT Neutralizing Antibody, PE-Labeled	71228	100 µg
TIGIT, Fc fusion, Avi-Tag, Biotin-labeled (Mouse) HiP™	79269	50 µg
Anti-TIGIT Inhibitor Antibody	71218	100 µg
TIGIT:CD112 Homogeneous Assay Kit	72030	384 reactions
TIGIT:CD155 Homogenous Assay Kit	72029	384 reactions
TIGIT, Fc fusion (Human)	71186	100 µg
TIGIT, Fc fusion, Biotin labeled (Human) HiP™	71251	50 µg
CD155/TCR Activator -CHO recombinant cell line	60548	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml

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