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Data Sheet

TIGIT / NFAT Reporter - Jurkat Recombinant Cell Line
Catalog #: 60538

Product Description
Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of human TIGIT (V-set and immunoglobulin domain-containing protein 9, VSIG9, V-set and transmembrane domain-containing protein 3, and VSTM3, GenBank Accession No. 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-kB 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
- Screen for activators or inhibitors of TIGIT signaling in a cellular context
- Characterize the biological activity of TIGIT and its interactions with ligands

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 PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

Culture Medium and Recommended Culture Conditions

Thaw Medium 2 (BPS Bioscience Cat. #60184): RPMI 1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01).

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Growth Medium 2A (BPS Bioscience Cat. #60190): Thaw Medium 2 (BPS Bioscience Cat. #60184) + 1 mg/ml of Geneticin (Life Technologies #11811031) and 200 µg/ml of Hygromycin B (Hyclone #SV30070.01).

Cells should be grown at 37°C with 5% CO_2 using **Growth Medium 2A.**

If culturing cells in medium from other vendors, it may be required to adjust the percentage of CO_2 in the incubator depending on the $NaHCO_3$ level in the basal medium.

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 (**no Geneticin, no Hygromycin B)**, spin down cells at 1500 rpm, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 2 (**no Geneticin, 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, add an additional ~3 ml of fresh warm Thaw Medium 2 (**no Geneticin, 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 2.5 x10^6 cells/ml. At first passage switch to Growth Medium 2A (**contains Geneticin and Hygromycin B)**.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml. Subcultivation ratio: ~1:10 twice a week, so cells are maintained at 0.2 x 10^6 cells/ml to 2.5 x10^6 cells/ml.

Note: Just after thawing, the cells may grow at a slower rate. It is recommended to split the cells at no less than 0.4 x10^6 cells/ml *at the beginning of culturing. After ~two passages, the cell growth rate increases and the cells can be split to 0.2x10^6 cells/ml.*

To freeze down the cells, spin down cells, and resuspend cell pellet 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 at early passage freeze down more than 10 vials of cells for future use.

Functional Validation and Assay Performance
Expression of human TIGIT in Jurkat cell line was confirmed by FACS.

The functionality of the cell line was validated using a TIGIT:CD155 cell-based assay. In this assay, TIGIT/NFAT Reporter/Jurkat T cells are used as effector cells; CHO cells over-expressing CD155 and an engineered T cell receptor (TCR) activator are used as target cells. When these two cells are co-cultivated, NFAT luciferase reporter in effector cells is activated by the engagement of TCR complexes with TCR activator on target cells and the engagement of the co-stimulatory receptor CD226 with CD155 on target cells. However, TIGIT can compete with CD226 for binding to CD155, therefore, TIGIT and CD155 ligation suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by anti-TIGIT

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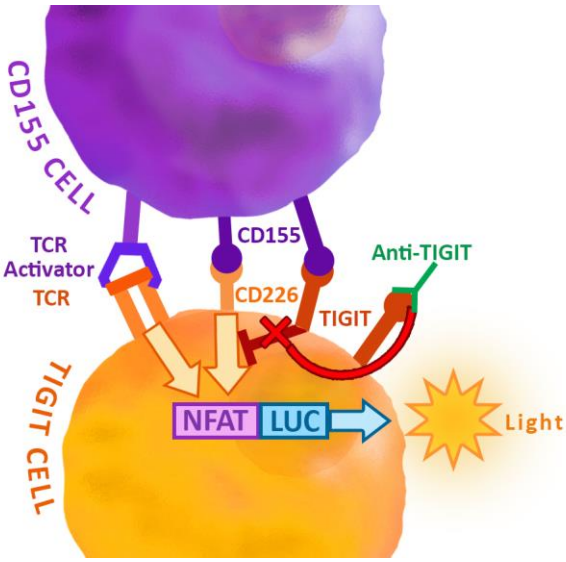
neutralizing antibody. The neutralizing antibody blocks TIGIT:CD155 interaction and promote T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter.



Materials Required for Culture but Not Supplied

- Thaw Medium 2 (BPS Bioscience Cat. #60184)
- Growth Medium 2A (BPS Bioscience Cat. #60190)

Materials Required for Assay but Not Supplied

- CD155/TCR Activator-CHO cell line (BPS Bioscience # 60548)
- Assay Medium 3A (BPS Bioscience #79816): Ham's F12 + **1% FBS** + 1% Penicillin/Streptomycin
- Anti-TIGIT neutralizing antibody (BPS Bioscience # 71340)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience # 60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

1. Harvest CD155/TCR Activator-CHO cells from culture and seed cells at a density of 2,500 cells per well into white clear-bottom 96-well microplate in 100 µl of Assay Medium 3A (BPS Bioscience #79816) (no Geneticin and Puromycin). Incubate cells at 37° in a CO2 incubator for overnight.

2. Next day, dilute anti-TIGIT antibody in Assay Medium 3A (the concentration of antibody here is 2x of the final treatment concentration of antibody).

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Harvest the TIGIT/NFAT-reporter-Jurkat cells by centrifugation, wash once with PBS and resuspend in Assay Medium 3A. Dilute cells to $4x10^5$ / ml in Assay Medium 3A. Preincubate the TIGIT/NFAT Reporter- Jurkat cells ($4x10^5$ / ml) with diluted anti-TIGIT antibody (1:1 in volume) for 30 min at 37° in a CO2 incubator. After incubation, remove the medium from CD155/TCR Activator-CHO cells and add 100 µl of TIGIT/NFAT reporter – Jurkat cells / anti-TIGIT antibody mixture to the wells. (Note: *Mix the TIGIT/NFAT Reporter- Jurkat cells with antibody well before adding to CD155/TCR Activator-CHO cells.*)

Final cell density of TIGIT/NFAT Reporter- Jurkat cells is 2 $x10^4$ /well. Set up each treatment in at least triplicate.

Add 100 µl of Assay Medium 3A to cell-free control wells (for determining background luminescence).

Incubate the plates at 37° in a CO2 incubator for 5 to 6 hours.

3. After ~5 to 6 hour incubation, perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of One-Step Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer.
   If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

4. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
   The fold induction of NFAT luciferase reporter expression = background-subtracted luminescence of treated well / average background-subtracted luminescence of untreated control wells.

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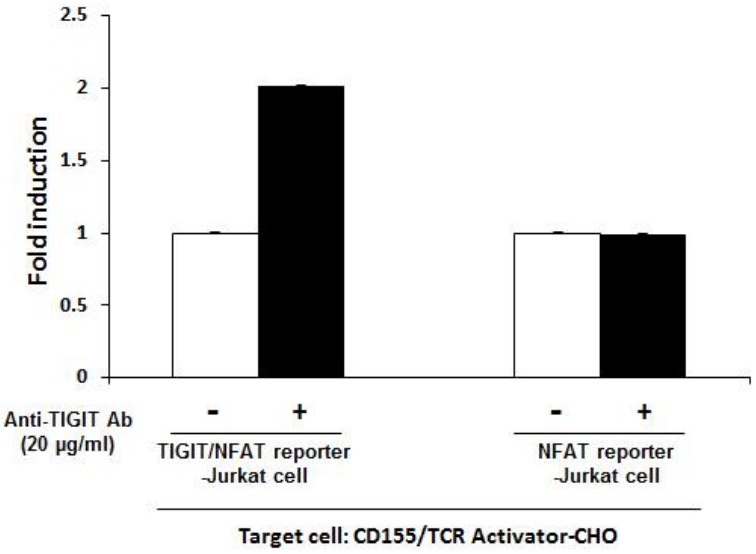
Figure 1. Anti-TIGIT neutralizing antibody induced the NFAT luciferase reporter activity in TIGIT/NFAT Reporter-Jurkat cells co-cultured with CD155/TCR Activator-CHO cells.
CD155/TCR Activator-CHO cells (BPS Bioscience # 60548) or TCR Activator-CHO cells (BPS Bioscience # 60539) were seeded in 96-well plate. The next day, cells were incubated with anti-TIGIT neutralizing antibody (BPS Bioscience # 71340) and TIGIT/NFAT Reporter-Jurkat cells or control NFAT Reporter – Jurkat cells (BPS Bioscience Cat. #60621). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience Cat. #60690) was added to the cells to measure NFAT activity.

The fold induction is equal to background-subtracted luminescence of antibody-treated well/background-subtracted luminescence of untreated-control wells of each respective cell line.

A. Anti-TIGIT neutralizing antibody induced the NFAT luciferase reporter activity in TIGIT/NFAT Reporter-Jurkat cells, but not in control NFAT Reporter-Jurkat cells, when co-cultured with CD155/TCR Activator-CHO cells



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B. Anti-TIGIT neutralizing antibody induced the NFAT luciferase reporter activity in TIGIT/NFAT Reporter-Jurkat cells when co-cultured with CD155/TCR Activator-CHO cells, but not with control TCR Activator-CHO cells.

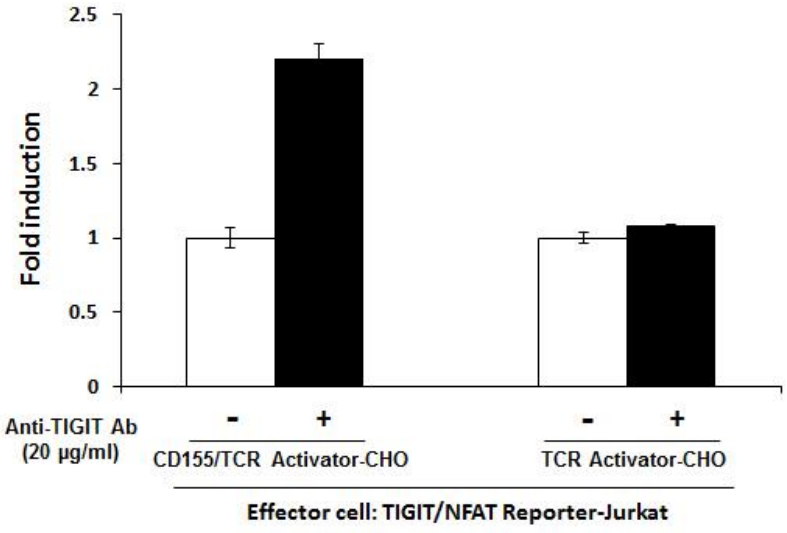

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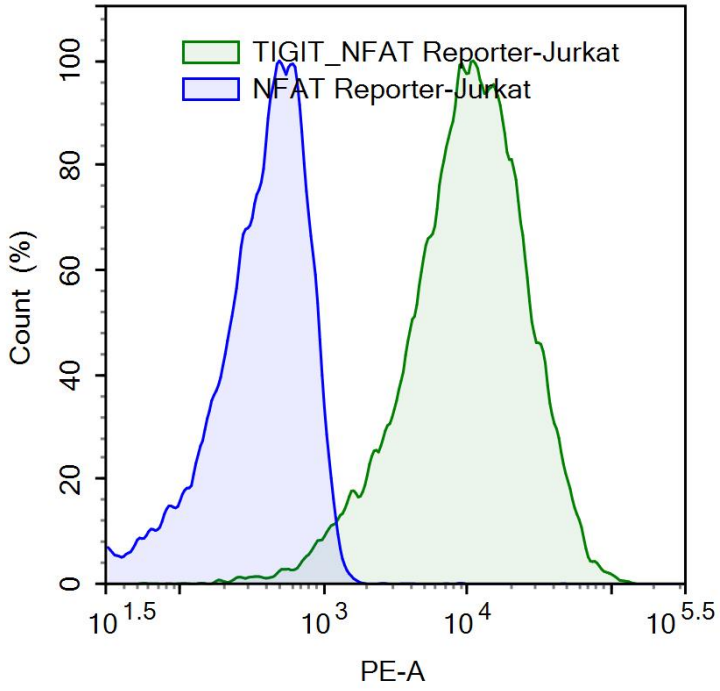
Figure 2. FACS analysis of cell surface expression of TIGIT in TIGIT/NFAT Reporter-Jurkat cells.
TIGIT/NFAT Reporter-Jurkat cells or control NFAT Reporter-Jurkat cells were stained with PE-labeled anti-TIGIT antibody (BPS Bioscience Cat #71228) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.



	Samples	Cell Count
	NFAT reporter-Jurkat	16224
	TIGIT/NFAT-Jurkat	16451

Sequence

TIGIT sequence (accession number NM_173799)

MRWCLLLIWAQGLRQAPLASGMMTGTIETTGNISAEKGGSIILQCHLSSTTAQVTQVNWEQQDQLLAICN
ADLGWHISPSFKDRVAPGPGLGLTLQSLTVNDTGEYFCIYHTYPDGTYTGRIFLEVLESSVAEHGARFQI
PLLGAMAATLVVICTAVIVVVALTRKKKALRIHSVEGDLRRKSAGQEEWSPSAPSPPGSCVQAEAAPAGL
CGEQRGEDCAELHDYFNVLSYRSLGNCSFFTETG*

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Related Products

Product	Cat. #	Size
CD155/TCR Activator -CHO recombinant cell line	60548	2 vials
TCR activator-CHO cell line	60539	2 vials
NFAT Reporter – Jurkat cell line	60621	2 vials
Thaw Medium 2	60184	100 ml
Anti-TIGIT neutralizing antibody	71340	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Anti-TIGIT Antibody, PE-labeled	71228-1	50 µg
Anti-TIGIT Antibody, PE-labeled	71228-2	100 µg
Human CD155 (PVR) His-tag Protein	71181	100 µg
Mouse CD155 (PVR) His-tag Protein	71167	100 µg
Mouse CD155, His-tag, Biotin-labeled	71168	50 µg
Human CD226, Fc fusion	71252	100 µg
Human CD226, Fc fusion, Biotin-labeled	71253	50 µg
Human CD112, Fc fusion	71197	100 µg
Human CD112, Fc fusion, Biotin-labeled	71234	50 µg
Human TIGIT, Fc fusion	71186	100 µg
Human TIGIT, Fc fusion, Biotin-labeled	71251	50 µg
TIGIT:CD155 Homogenous Assay Kit	72029	384 reactions
CD226: CD155 Homogenous Assay Kit	72052	384 reactions
TIGIT:CD112 Homogenous Assay Kit	72030	384 reactions
CD226: CD112 Homogenous Assay Kit	72051	384 reactions

Notes

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