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# Data Sheet

## CD155 / TCR Activator - CHO Recombinant Cell line
## Cat. #: 60548

**Product Description**
Recombinant CHO-K1 cells constitutively expressing human CD155 (Poliovirus receptor, Nectin-like protein 5, GenBank accession # NM_006505) and an engineered T cell receptor (TCR) activator.

**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 phosphatases 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. TIGIT serves as a competitive inhibitor of CD226, a co-stimulatory receptor for CD155. TIGIT-targeting antibodies that block these T cell-intrinsic inhibitory effects have shown enhanced anti-tumor and anti-viral functions in preclinical studies.

**Applications**
- Screen for activators or inhibitors of TIGIT signaling in a cellular context
- Screen CD155 antibodies for binding affinity
- Characterize the biological activity of TIGIT or CD226 interactions with CD155

**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, #MP0025)) to confirm the absence of *Mycoplasma* species.

**General Culture Conditions**

**Thaw Medium 3 (BPS Bioscience, #60186):** F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

**Growth Medium 3C (BPS Bioscience, #79537):** F-12K Medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin B and 5 µg/ml of Puromycin

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Cells should be grown at 37°C with 5% $CO_2$ using the Growth Medium 3C to ensure recombinant expression. CD155 / TCR activator– CHO cells should exhibit a typical cell division time of ~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 3 (**no Hygromycin B, no Puromycin)**, spin down cells at 1000 rpm, and resuspend cells in 5 ml of pre-warmed Thaw Medium 3 (**no Hygromycin B, no Puromycin)**. 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 3 (**no Hygromycin B, no Puromycin)**, 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 3C (**contains Hygromycin B and Puromycin)**.

**To passage the cells**, rinse cells with phosphate buffered saline (PBS), then detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Growth Medium 3C and transfer to a tube. Spin down cells, resuspend cells in Growth Medium 3C (**contains Hygromycin B and Puromycin)** and seed appropriate aliquots of cell suspension into a new culture vessel. Sub cultivation ration: 1:10 to 1:20 twice a week.

**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 3 (**no Hygromycin B, no Puromycin)** 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 for future use at an early passage.

**Functional Validation and Assay Performance**
Expression of human CD155 in CD155/TCR Activator-CHO cells 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 (BPS Bioscience, #60538) are used as effector cells; the CD155/TCR Activator - CHO cells are used as target cells. When these two cells are co-cultivated, NFAT luciferase reporter in the effector cells is activated by the engagement of TCR complexes with TCR activator on the 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 binding suppresses the NFAT-responsive luciferase activity. This inhibition can be specifically reversed by anti-TIGIT neutralizing antibody. The neutralizing antibody blocks TIGIT:CD155 interaction and promotes T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter.

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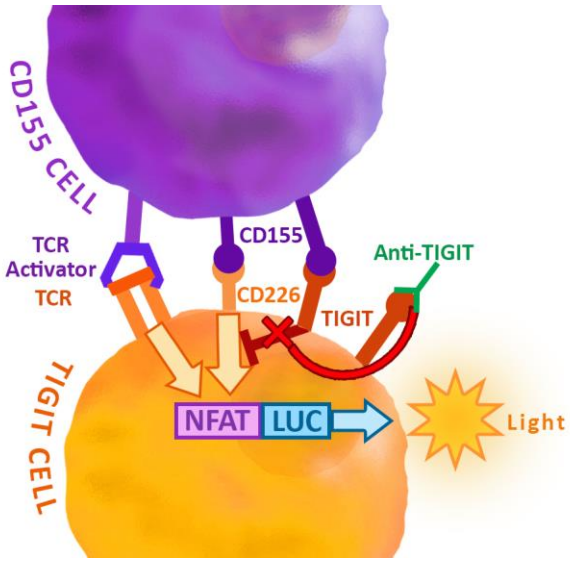


**Materials Required but Not Supplied for Cell Culture**

- Thaw Medium 3 (BPS Bioscience Cat. #60186): Ham's F-12 medium (Hyclone, #SH30526.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).
- Growth Medium 3C (BPS Bioscience, #79537)

**Materials Required but Not Supplied for Cellular Assay**

- TIGIT/NFAT Reporter - Jurkat Cell Line (BPS Bioscience # 60538)
- Assay Medium 3A (BPS Bioscience #79816): Ham's F12 medium + **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 µls 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).

   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

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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 x$10^4$ /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.

**Figure 1. Anti-TIGIT neutralizing antibody induced NFAT luciferase reporter activity in TIGIT/NFAT Reporter-Jurkat cells co-cultured with CD155/TCR Activator-CHO cells.**
CD155/TCR Activator-CHO cells 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 (BPS Bioscience, #60538) or control NFAT Reporter – Jurkat cells (BPS Bioscience, #60621). After incubation, ONE-Step™ Luciferase reagent (BPS Bioscience, #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 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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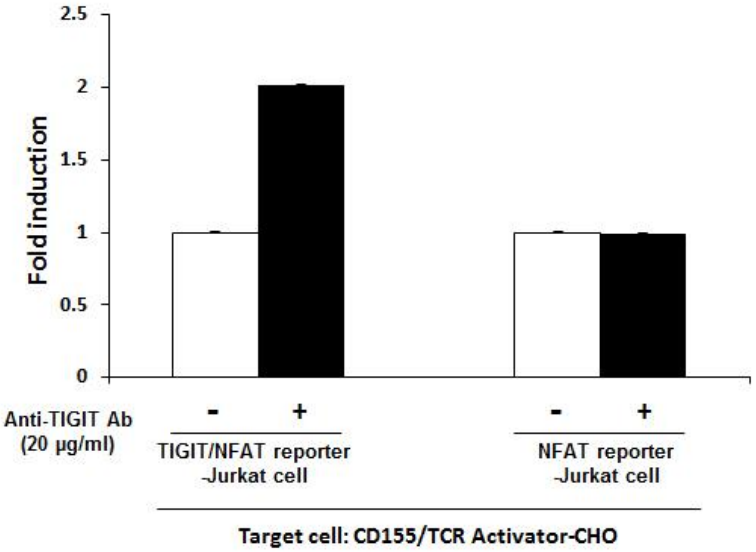


B. Anti-TIGIT neutralizing antibody induced NFAT luciferase reporter activity in TIGIT/NFAT Reporter-Jurkat cells when co-cultured with CD155/TCR Activator-CHO cells, but not with control TCR Activator-CHO cells.

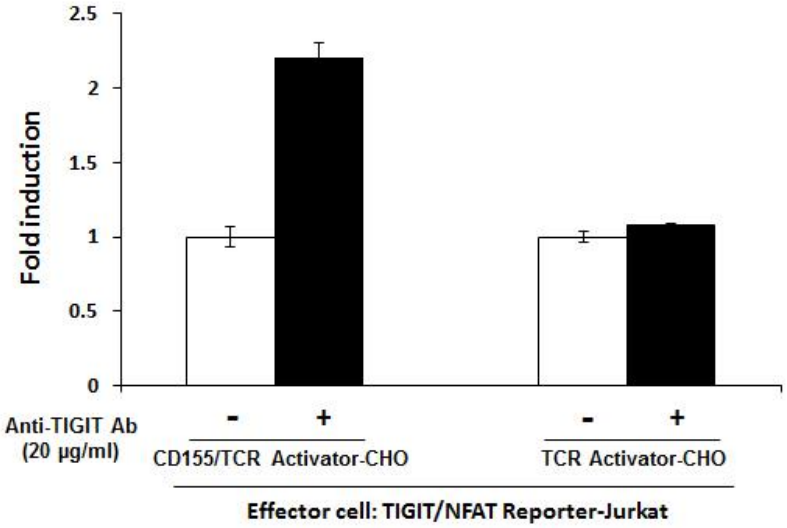


**Figure 2. FACS analysis of cell surface expression of CD155 in CD155/TCR Activator-CHO cells.**
CD155/TCR Activator cells or control CHO cells were stained with PE-labeled anti-CD155 antibody (Biolegend, #337610) and analyzed by FACS. Y-axis is the % cell number. X-axis is the intensity of PE.

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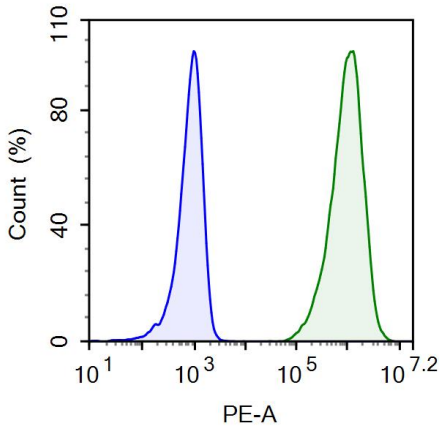


| | Samples | Subset | Cell Count |
|---|---|---|---|
| | CD155/TCR Activator-CHO | Live singlet | 10207 |
| | Control CHO | Live singlet | 10116 |

## Sequence

Human CD155 sequence (accession #NM_006505)

MARAMAAAWPLLLVALLVLSWPPPGTGDVVVQAPTQVPGFLGDSVTLPCYLQVPNMEVTHVSQL
TWARHGESGSMAVFHQTQGPSYSESKRLEFVAARLGAELRNASLRMFGLRVEDEGNYTCLFVTF
PQGSRSVDIWLRVLAKPQNTAEVQKVQLTGEPVPMARCVSTGGRPPAQITWHSDLGGMPNTSQV
PGFLSGTVTVTSLWILVPSSQVDGKNVTCKVEHESFEKPQLLTVNLTVYYPPEVSISGYDNNWY
LGQNEATLTCDARSNPEPTGYNWSTTMGPLPPFAVAQGAQLLIRPVDKPINTTLICNVTNALGA
RQAELTVQVKEGPPSEHSGMSRNAIIFLVLGILVFLILLGIGIYFYWSKCSREVLWHCHLCPSS
TEHASASANGHVSYSAVSRENSSSQDPQTEGTR

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## Related Products

| Product | Cat. # | Size |
|---|---|---|
| TIGIT/NFAT Reporter-Jurkat recombinant cell line | 60538 | 2 vials |
| TCR activator-CHO cell line | 60539 | 2 vials |
| NFAT Reporter – Jurkat cell line | 60621 | 2 vials |
| Thaw Medium 2 | 60184 | 100 ml |
| Thaw Medium 3 | 60186 | 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 |
| 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 |

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