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Data Sheet

GITRL CHO-K1 Recombinant Cell Line
Catalog # 60547

Background
GITRL (CD357; AITRL; TNLG2A), a part of the tumor necrosis factor (ligand) superfamily, member 18 (TNFSF18), is mainly expressed on antigen presenting cells. GITRL engages with the GITR receptor as a co-stimulatory signal to promote lymphocyte activation.

Description
Recombinant CHO-K1 cell constitutively expressing full length human GITRL (NP_005083.2). Surface expression is confirmed by flow cytometry.

Host Cell
Chinese Hamster Ovary Cells. Adherent epithelial cells.

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

Storage
Store in liquid nitrogen immediately upon receipt.

Application
This cell line is useful for stimulating human GITR on immune cells, as well as binding assays for GITR. BPS Bioscience has validated this cell line stimulates NF-kB activation using the GITR NF-kB-Luciferase cell line (BPS Bioscience, Cat. # 60546).

Culture Medium

Thaw Medium 3 (BPS 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 3D (BPS Cat. #79539): Thaw Medium 3 (BPS Cat. #60186) plus 1 mg/ml G418 (Thermo Fisher, Cat. #11811031).

Recommended Culture Condition
Prepare a 50 ml conical tube and a T-25 culture flask with 5 ml of pre-warmed Thaw Medium 3 **(no G418)**. Quickly thaw cells in a 37°C water bath with constant and slow agitation. Clean the outside of the vial with 70% ethanol and immediately transfer the entire contents to the conical tube with Thaw Medium 3 **(no G418)** and rock the tube the tube gently. Centrifuge the cells at 200 x g for 3 minutes. Re-suspend the cells in 5 ml of pre-warmed Thaw Medium 3 and transfer the entire content to the T25 culture flask containing Thaw Medium 3 **(no G418)**. Avoid pipetting

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up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2.

24-48 hours after incubation, change to fresh media without disturbing the attached cells. Continue to change Thaw Medium 3 **(no G418)** every 2-3 days until cells reach desired confluency. If experiencing slow cell growth during resuscitation, use 15% FBS during the first week of culture.

Subculture: When cells reach 90% confluency, remove the media and wash twice with PBS (without Magnesium or Calcium). Treat cells with 2-3 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by a light microscope, add 10 ml of pre-warmed Growth Medium 3D and gently pipette up and down to dissociate cell clumps. Transfer cells to a 15 ml conical tube and centrifuge at 200 x g for 5 minutes. Remove the media and resuspend cells in 10 ml of pre-warmed Growth Medium 3D **(contains G418).** Dispense 2 ml of the cell suspension into a new T75 flask containing pre-warmed 18 ml Growth Medium 3D. Incubate cells in a humidified incubator as mentioned above. Freeze cells in freezing media (10% DMSO in FBS) when they reach 90% confluency. It is not recommended to use the cells after passage 20.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, Cat. # LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, Cat. # LT07-518) was used as a positive control.

Quality Assurance

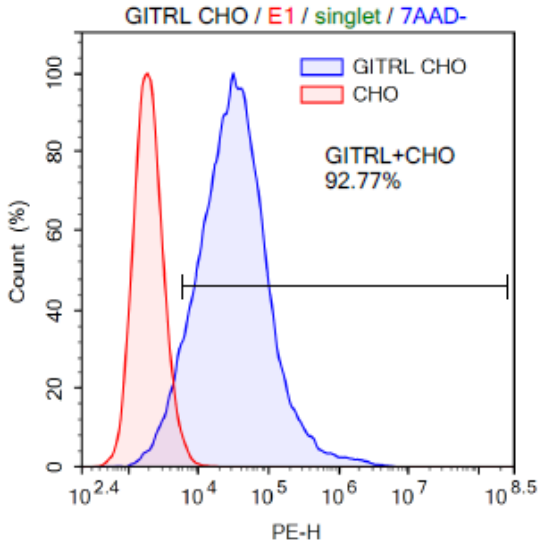

Figure 1. Human GITRL expression in CHO-K1 cells

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Flow cytometry demonstrates PE conjugated anti-human GITRL antibody (R&D Systems, Cat. #FAB6941P) detects GITRL-positive cells (blue), using CHO-K1 cells as a negative control (red).

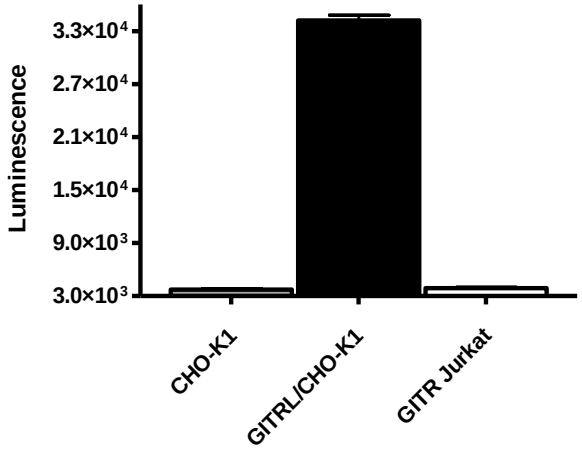

Figure 2. GITRL CHO-K1 cells stimulate GITR NF-κB-Luciferase Jurkat. GITRL CHO-K1 or CHO-K1 cells were seeded overnight at 1-3 x 10^4 cells/well. The next day, 3 x 10^4 GITR NF-κB Jurkat cells were added to GITRL CHO-K1 (black bar), CHO-K1 cells (gray bar), or incubated alone (white bar) in serum-free RPMI for 3-4 hours. ONE-Step™ Luciferase Assay System was added to each well, according to recommended protocol. Data was analyzed by GraphPad Prism®. Error bar = SEM; n = 6.

Application Reference

Hanabuchi S *et.al.* (2006). Human plasmacytoid predendritic cells activate NK cells through glucocorticoid-induced tumor necrosis factor receptor-ligand (GITRL). *Blood* **107:** 3617-3623

Vector and Sequence

Human GITRL (NM_005092.3) was cloned into the MCS of pIRESneo3 vector (Clontech, Cat. #631621).

AA Sequence

MTLHPSPITCEFLFSTALISPKMCLSHLENMPLSHSRTQGAQRSSWKLWLFCSIVMLLFLCSFS
WLIFIFLQLETAKEPCMAKFGPLPSKWQMASSEPPCVNKVSDWKLEILQNGLYLIYGQVAPNAN
YNDVAPFEVRLYKNKDMIQTLTNKSKIQNVGGTYELHVGDTIDLIFNSEHQVLKNNTYWGIILLA
NPQFIS

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Related Products	**Cat. #**	**Size**
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
ONE-Step™ Luciferase Assay System	60690-2	1 L
GITR / NF-κB-Luciferase Reporter (Luc) - Jurkat Cell Line	60546	2 vials
GITRL, His-tag (Human)	71190	100 µg
GITR (CD357), Fc fusion (Human) HiP™	71172	100 µg
GITR (CD357), Fc fusion, Biotin-labeled (Human)	71256	50 µg
GITRL:GITR[Biotinylated] Inhibitor Screening Assay Kit	72061	96 rxns.
Anti-GITR Antibody, PE-labeled	71295-1	50 µg
Anti-GITR Antibody, PE-labeled	71295-2	100 µg
Thaw Medium 3	60186	100 ml