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Data Sheet

GITR - HEK293 Recombinant Cell Line
Catalog # 79092

Background
This cell line expresses human GITR (glucocorticoid-induced TNFR family-related gene; TNFRSF18; CD357), Genbank Accession Number NM_ 004195.2.

Description
Recombinant HEK293 cell constitutively expressing full length human GITR. Surface expression is confirmed by flow cytometry.

Host Cell
Human Embryonic Kidney cell line (HEK293). 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.

Culture Medium

Thaw Medium 1 (BPS Bioscience, #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Invitrogen #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 (BPS Bioscience #60187) plus 100 µg/ml Hygromycin B (Thermo Fisher, #10687010).

Recommended Culture Condition
Frozen Cells: Prepare a 50 ml conical tube with 10 ml of pre-warmed Thaw Medium 1 (**no hygromycin**). 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 content to Thaw Medium 1 (**no hygromycin**). Avoid pipetting up and down, and gently rock the conical tube.

Spin the cells down at 150 x g for 5 minutes. Discard the medium and re-suspend the cell pellet in fresh Thaw Medium 1 (**no hygromycin**). Transfer the entire content to a T25 flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO_2. After 48-72 hours of incubation, change to fresh Thaw Medium 1 (**no hygromycin**), without disturbing the attached cells. Continue to change the medium every 2-3 days until the cells reach desired

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confluency. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Switch to Growth Medium 1F (**containing hygromycin**) after the first passage.

Subculture: When cells reach 90% confluency, remove the medium and GENTLY wash once with PBS (without Magnesium or Calcium). These cells are loosely adherent and detach easily so do not re-suspend the PBS directly onto the cell surface. Treat cells with 2 ml of 0.25% trypsin/EDTA and incubate for 2-3 minutes at 37°C. After confirming cell detachment by light microscopy, add 10 ml pre-warmed Growth Medium 1F 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 medium and re-suspend cells in 10 ml of pre-warmed Growth Medium 1F. Dispense 5 ml of the cell suspension into a new T75 flask containing pre-warmed 20 ml Growth Medium 1F. Incubate cells in a humidified 37°C incubator with 5% CO_2. Freeze cells in freezing medium (10% DMSO in FBS) when cells reach 90% confluency. Cells have been demonstrated to be stable for at least 15 passages; BPS recommends preparing frozen stocks so cells are not used beyond passage 20.

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

Application
This cell line is a good candidate for binding analyses and for studying GITR receptor activity.

References
1. Ronchetti, S., *et al. Eur J Immunol*. 2004; **34(3):**613-22.
2. Mahne AE, *et al. Cancer Res*. 2017; **77(5):**1108-1118.

Quality Assurance

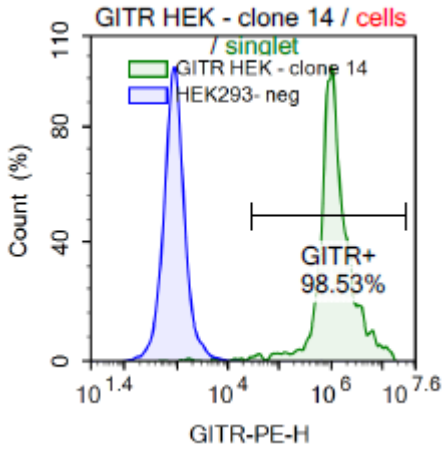

Figure 1. Expression of GITR protein in GITR HEK293 validated by flow cytometry. Flow cytometry showed PE-conjugated anti-human GITR antibody (BPS Bioscience, #71295) detects GITR-positive cells (green), using wild-type HEK293 cells as a negative control (blue).

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Vector and Sequence
GITR sequence (NM_ 004195.2) was cloned into pIREShyg.

AA Sequence:
MAQHGAMGAFRALCGLALLCALSLGQRPTGGPGCGPGRLLLGTGTDARCCRVHTTRCCRD
YPGEECCSEWDCMCVQPEFHCGDPCCTTCRHHPCPPGQGVQSQGKFSFGFQCIDCASGTF
SGGHEGHCKPWTDCTQFGFLTVFPGNKTHNAVCVPGSPPAEPLGWLTVVLLAVAACVLLL
TSAQLGLHIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFPEEERGERSAEEKGRLGDLWV

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Related Product	**Cat. #**	**Size**
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
GITR / NF-κB Luciferase Reporter (Luc) - Jurkat Cell Line	60546	2 vials
GITRL CHO-K1 Recombinant Cell Line	60547	2 vials
Anti-GITR Antibody, PE-labeled	71295-2	100 µg
GITRL:GITR[Biotinylated] Inhibitor Screening Assay Kit	72061	96 rxns
GITR (CD357), Fc fusion (Human) HiP™	71172	100 µg
GITR (CD357), Fc Fusion, Biotin-labeled (Human)	71256	50 µg
GITRL, His-tag (Human)	71190	100 µg