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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<sup>6</sup> 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<sub>2</sub>. 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<sub>2</sub>. 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.

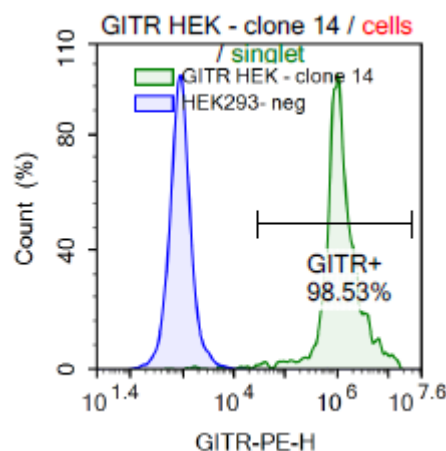
### Application

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



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**Figure 1. Expression of G1TR protein in G1TR HEK293 validated by flow cytometry.** Flow cytometry showed PE-conjugated anti-human G1TR antibody (BPS Bioscience, #71295) detects G1TR-positive cells (green), using wild-type HEK293 cells as a negative control (blue).

#### Vector and Sequence

G1TR sequence (NM\_004195.2) was cloned into pIRESHyg3.

AA Sequence:

MAQHGMGAFRALCGLALLCALSLGQRPTGGPGCGPGRLLLGTGTDARCCRVHTTRCCRD  
YPGEECCSEWDCMCVQPEFHCGDPCCTTCRHHPCPPGQGVQSQGKFSFGFCIDCASGTF  
SGGHEGHCKPWTDCQFGFLTVPNGKTHNAVCPGSPPAEPLGWLTVLLAVAACVLLL  
TSAQLGLHIWQLRSQCMWPRETQLLLEVPPSTEDARSCQFP EEER GERSAEEKGRLGLDWV

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#### Related Product

	<b>Cat. #</b>	<b>Size</b>
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
G1TR / NF-κB Luciferase Reporter (Luc) - Jurkat Cell Line	60546	2 vials
G1TRL CHO-K1 Recombinant Cell Line	60547	2 vials
Anti-G1TR Antibody, PE-labeled	71295-2	100 µg
G1TRL:G1TR[Biotinylated] Inhibitor Screening Assay Kit	72061	96 rxns
G1TR (CD357), Fc fusion (Human) HiP™	71172	100 µg
G1TR (CD357), Fc Fusion, Biotin-labeled (Human)	71256	50 µg
G1TRL, His-tag (Human)	71190	100 µg

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