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## Data Sheet

### **GITR / NF- $\kappa$ B-Luciferase Reporter (Luc) - Jurkat Cell Line**

### **Catalog #60546**

#### **Description**

This cell line expresses a surface human GITR (glucocorticoid-induced TNFR family related gene; TNFRSF18; CD357) and an NF- $\kappa$ B luciferase reporter construct that are stably integrated into the genome of Jurkat T-cells. The firefly luciferase gene is controlled by 4 copies of NF- $\kappa$ B response element located upstream of the TATA promoter. Following activation by stimulants, endogenous NF- $\kappa$ B transcription factors bind to the DNA response elements to induce transcription of the luciferase gene.

#### **Host Cell**

Human Acute T-Cell Leukemic Cell Line (Clone E61). Suspension cells.

#### **Format**

Each vial contains  $\sim 2 \times 10^6$  cells in 1 ml of 10% DMSO in FBS.

#### **Storage**

Store in liquid nitrogen immediately upon receipt.

#### **General Culture Conditions**

**Thaw Medium 2 (BPS Cat. #60184):** RPMI1640 medium (Life Technologies, #A10491-01) supplemented with 10% FBS (Life Technologies, #26140-079), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

**Growth Medium 2C (BPS Cat. #79592):** Thaw Medium 2 (BPS Cat. #60184), plus 100  $\mu$ g/ml Hygromycin (Thermo Fisher, #10687010) and 1 mg/ml G418 (Thermo Fisher, #11811031).

#### **Recommended Culture conditions**

*Frozen Cells:* Prepare a 50 ml conical tube and a T-75 culture flask with 10 ml of pre-warmed Thaw Medium 2. 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 2 (**without G418 & Hygromycin**) and rock 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 2 and transfer the entire content to the T75 culture flask containing Thaw Medium 2 (**without G418 & Hygromycin**). Avoid pipetting up and down, and gently rock the flask to distribute the cells. Incubate the cells in a humidified 37°C incubator with 5% CO<sub>2</sub>. Forty-eight hours after incubation, centrifuge cells at 250 x g for 5 minutes and re-suspend to Thaw Medium 2 (**without G418 & Hygromycin**). Continue to monitor growth for 2-3 days and change medium to remove dead debris. If slow cell growth occurs during resuscitation, increase FBS to 15% for the first week of culture. Switch to Growth Medium 2C (**containing G418 & Hygromycin**) after multiple cell colonies (in clumps) start to appear (indicative of healthy cell division). We

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recommend passing cells for 3 passages after thawing before using them in the luciferase assay.

*Subculture:* When cells reached 90% confluency, transfer cells to a 50 ml conical tube and centrifuge cells at 200 x g for 5 minutes. Wash cells once with PBS (without Magnesium or Calcium) and re-suspend cells in 10 ml pre-warmed Growth Medium 2C; gently pipette up and down to dissociate cell clumps. Dispense 2 ml of the cell suspension into a new T-75 flask containing pre-warmed 15-20 ml Growth Medium 2C. 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 (Cat. #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Cat. #LT07-518) was used as a positive control.

### **Application**

The GITR NF-κB-luciferase/Jurkat cell line is suitable for monitoring the activity of NF-κB signaling in response to GITR ligand (GITRL). This reporter cell line has been tested and validated by BPS using purified human GITRL (Figure 2, 3). We do not recommend starving the cells overnight in Thaw Medium 2 prior to stimulation, or treating cells beyond 5 hours incubation.

### **Application Reference**

1. Esparza, E.M. and Arch, R.H. (2005) Glucocorticoid-Induced TNF Receptor, a Costimulatory Receptor on Naïve and Activated T Cells, Uses TNF Receptor-Associated Factor 2 in a Novel Fashion as an Inhibitor of NF-κB Activation. *J Immunology*. **174**: 7875-7882.

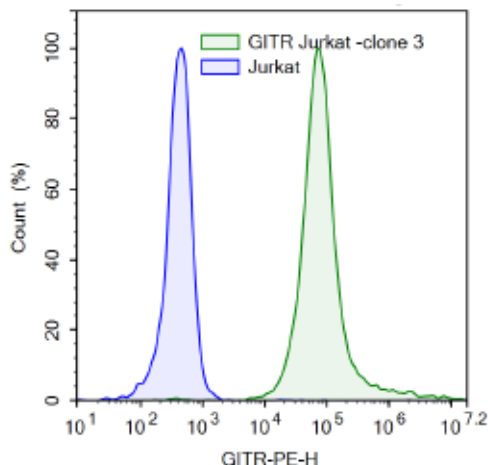
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## Quality Assurance



**Figure 1. Expression of GITR protein in GITR / NF- $\kappa$ B-Luciferase Reporter - Jurkat cells validated by flow cytometry.** Flow cytometry showed PE- conjugated anti-human GITR antibody (BPS Bioscience, Cat. #71295) detects GITR-positive cells (green), using wild-type Jurkat cells as a negative control (blue).

## Assay Protocol and Functional Analysis

### A) Analysis of GITR / NF- $\kappa$ B Jurkat reporter activity in response to purified human GITRL protein.

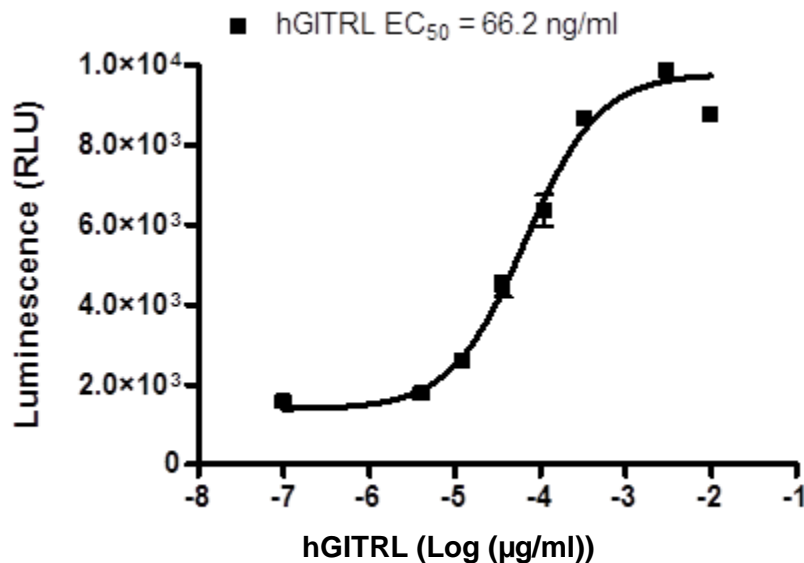
1. In a white opaque 96- well plate, seed cells at  $2 - 4 \times 10^4$  cells/well (100  $\mu$ l per well) in Thaw Medium 2 (BPS Cat. #60184). Cells should be growing at log phase at time of seeding.
2. Immediately treat cells with GITRL (detection range = 0.1 - 10  $\mu$ g/ml, final concentration) (Figure 2) for approximately 3 hours at 37°C with 5% CO<sub>2</sub>.
3. Add ONE-Step™ Luciferase Assay System (BPS Bioscience, Cat. #60690) to each well, according to recommended protocol.
4. Read luminescence using a luminometer. Normalize luminescence to wells that contain only medium to obtain the Relative Luminescence Units (RLUs).

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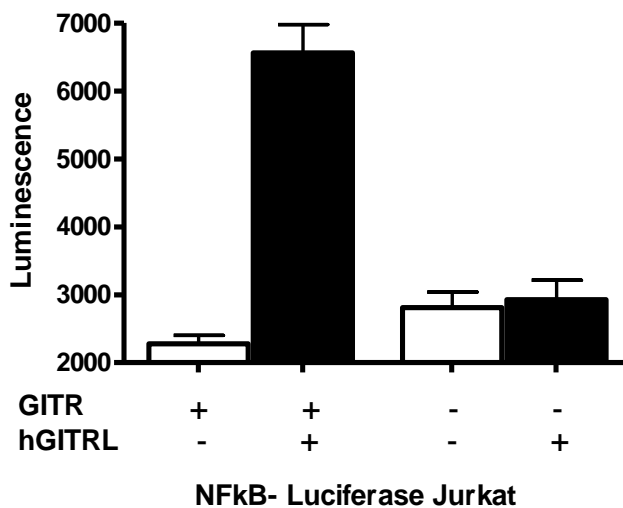
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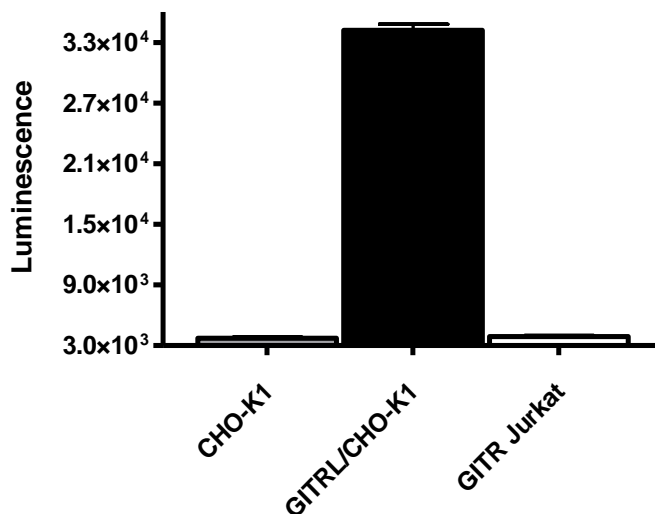


**Figure 2. Analysis of GITR NF-κB / Jurkat reporter activity in response to human GITRL.** Cells were seeded on a white opaque 96 well plate at  $4 \times 10^4$  cells / well (100ul per well) in Thaw Medium 2 and were immediately treated with human GITRL. Error bar = standard deviation (SD), n =3.



**Figure 3. GITRL stimulates GITR NF-κB Luciferase Jurkat, not NF-κB Luciferase.** Cells were treated with 1 µg/ml of human GITRL for 3 hours. Error bar = SEM, n=3.

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**Figure 4. GITR NFkB Reporter Cells recognizes GITRL presented in the CHO-K1 cells.** GITRL CHO or CHO cells were seeded overnight at  $1-3 \times 10^4$  cells /well. The next day,  $3 \times 10^4$  GITR NF-kB Jurkat cells were added to GITRL CHO (black bar), CHO-K1 cells (gray bar), or incubated alone (white) in Thaw Medium 2 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.

**B) Functional assay of anti-GITR agonist antibody on GITR / NF-kB Jurkat cells cocultured with FcGR2B CHO K1 cells**

1. Harvest GITR/NF-kB Jurkat cells from culture in growth medium and seed 30,000 cells per well into white clear-bottom 96-well plate in 50 µl of assay medium (Thaw Medium 2)
2. Harvest FcGR2B CHO K1 cells and seed 60,000 cells/well in 50ul of Thaw Medium 3 for coculture with the GITR/NF-kB Jurkat cells.
3. Immediately dilute anti-GITR agonist antibody (BPS cat #79053) in Thaw Medium 2 and treat cells with 10ul of 10X dilutions of the GITR antibody (for EC50 curve: 0.2-200ng/ml final concentration)
4. Incubate the plate at 37°C in a CO<sub>2</sub> incubator for about 5 hrs.
5. Perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of ONE-Step Luciferase reagent per well (BPS Bioscience, #60690) and rock at room temperature for 20 minutes. Measure luminescence using a luminometer
6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-kB

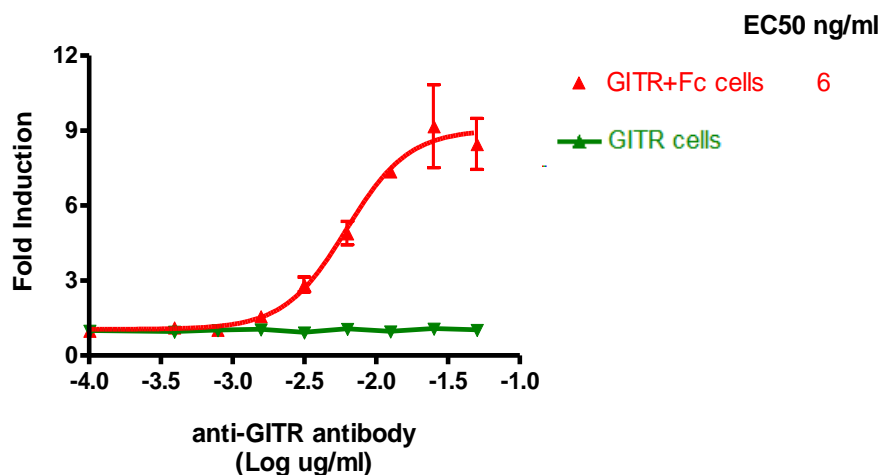
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luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.



**Fig. 5 Dose response of anti-GITR agonist antibody on GITR/NFkB Jurkat cells cocultured with FcGR2B CHO K1 cells**

GITR/NFkB Jurkat cells (BPS Cat. #60546) were cocultured with FcGR2B-CHO cells (BPS Cat# 79511), and serial dilutions of anti-GITR antibody were added to the cells. then incubated at 37°C incubator for 5 hrs. After the treatment, perform Luciferase assay

#### Vector

GITR sequence (NM\_004195.2)

MAQHGAMGAFRALCGLALLCALSLGQRPTGGPGCGPGRLLLGTGTDARCCRVTHTTRCCRD  
 YPGEECCSEWDCMCVQPEFHCGDPCCTTCRHHPCPPGQGVQSQGKFSFGFCIDCASGTF  
 SGGHEGHCKPWT DCTQFGFLTVFPGNKTHNAVCPGSPPAEPLGWLTVLLAVAACVLLL  
 TSAQLGLHIWQLRSQCMWPRETQLLLEVPSTEDARSCQFPPEEERGERSAEEKGRLGDLWV

NFkB-Luciferase was cloned into the MCS of pCDNA3.1™ (+) vector (Invitrogen, Cat. #V79020).

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Anti-GITR Antibody, PE-labeled	71295	100 µg
GITR Fc fusion protein	71172	100 µg
GITR Fc fusion protein, biotin-labeled	71256	50 µg
GITRL, His-tag protein	71190	100 µg
GITRL:GITR[Biotinylated] Inhibitor Screening Assay Kit	72061	96 rxns.
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml

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