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Data Sheet

GAS (IFNγ/JAK/STAT1 pathway) Reporter (Luc) – HeLa Cell Line

Catalog #: 79041

Product description
The GAS reporter (Luc)-HeLa cell line is designed to monitor the activity of interferon gamma-induced signal transduction pathways in cultured cells by measuring activated STAT1 homodimers. It contains a firefly luciferase gene driven by three copies of the interferon gamma-activated sites (GAS) located upstream of the minimal TATA promoter. IFNγ first binds to a heterodimeric receptor consisting of two chains, IFNGR1 and IFNGR2, causing its dimerization and the activation of specific Janus family kinases (JAK1 and JAK2). Two STAT1 molecules associate with this ligand-activated receptor complex and are activated by phosphorylation to form active homodimer. The active STAT1 homodimers translocate to the nucleus where they bind interferon gamma-activated sites (GAS) in the promoter of IFNγ inducible genes, including luciferase reporter gene.

Application
- Monitor interferon gamma-induced signal transduction pathways.
- Screen for activators or inhibitors of JAK/STAT1 signaling pathway.

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 1 (BPS Bioscience #60187): MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01)

Complete Growth Medium: Thaw Medium 1 (BPS Bioscience #60187) and 800 µg/ml of Geneticin (Life Technologies #11811031).

Cells should be grown at 37°C with 5% CO_2 using complete growth medium.

GAS reporter (Luc)-HeLa cells should exhibit a typical cell division time of 24 hours.

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To thaw the cells, it is recommended to quickly thaw the frozen cells directly from liquid nitrogen into a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 1 **(no Geneticin)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 1 **(no Geneticin)**. Transfer the resuspended cells to a T75 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 3 – 4 ml of Thaw Medium 1 **(no Geneticin)**. At first passage, switch to complete growth medium **(contains Geneticin)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, add complete growth medium and transfer to a tube. Spin down cells, resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ratio: 1:5 to 1:10, 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. Add complete growth medium and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and transfer to liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

Assay Performance
The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

Materials Required but Not Supplied

- IFNγ (PeproTech #300-02)
- IFNα (PBL Assay Science #11100-1)
- Assay medium: Thaw Medium 1 (BPS Bioscience, #60187) or
- MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS, 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone #SV30010.01)
- 96-well tissue culture treated white clear-bottom assay plate (Corning #3610)
- One-Step luciferase assay system (BPS Bioscience #60690)
- Luminometer

A. IFNγ dose response

1. Harvest GAS reporter (Luc)-HeLa cells from culture in growth medium and seed cells at a density of ~20,000 cells per well into white opaque 96-well microplate in 50 µl of assay medium. Incubate cells at 37°C with 5% CO_2 overnight.

2. Prepare threefold serial dilution of IFNγ in assay medium. Add 50 µl of diluted IFNγ to IFNγ -stimulated wells.

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Add 50 µl of assay medium to the unstimulated control wells (for measuring uninduced level of GAS reporter activity).

Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

5. Incubate at 37°C with 5% CO_2 for ~18-24 hours.

6. Prepare ONE-Step™ Luciferase Assay reagent as directed. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Figure 1. IFNγ dose response in GAS reporter (Luc)-HeLa cells. Cells were treated with IFNγ for ~ 18 hours. The results were shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without IFNγ treatment.

The EC50 of IFNγ in this cell line is ~1.0 ng/ml.

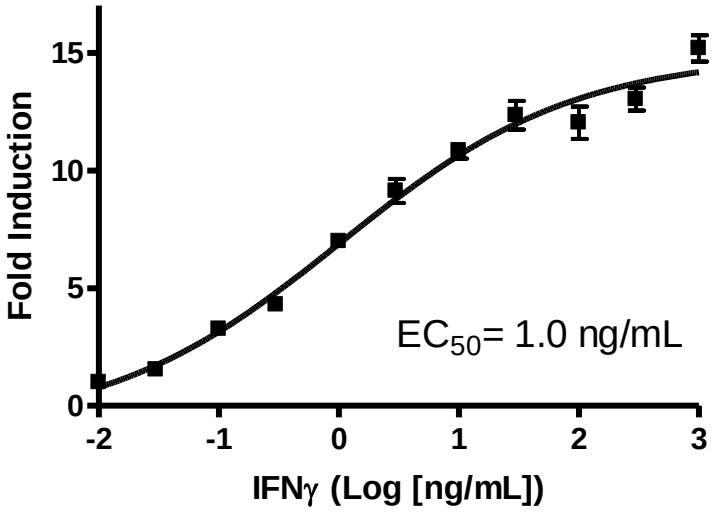

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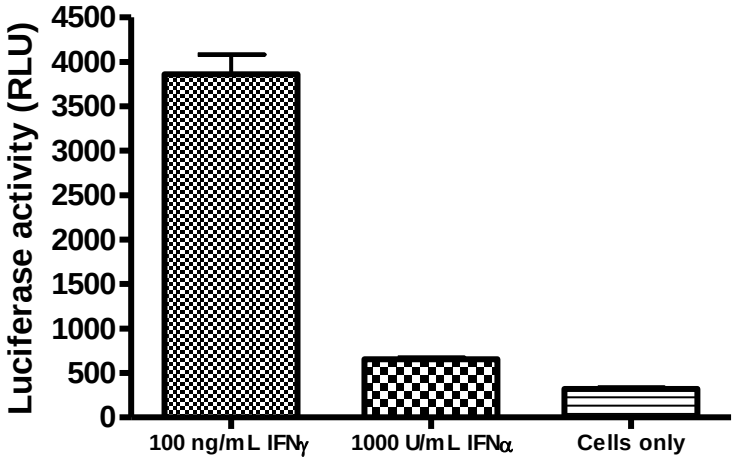
Figure 2. GAS reporter activity in response to IFNα. Cells were seeded at 20,000 cells/well on a white opaque 96-well plate overnight in assay medium before treatment with various human cytokines (IFNγ, 100 ng/ml; IFNα, 1000 ng/ml) and incubated for 18 hours, followed by the addition of luciferin according to manufacturer's protocol (ONE-Step™ Luciferase assay system, BPS Bioscience, #60690-2).



Related Products

Product	Cat. #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Human Interferon-gamma	90162-A	20 µg
Human Interferon-gamma	90162-B	100 µg
Mouse Interferon-gamma	90163-A	20 µg
Mouse Interferon-gamma	90163-B	100 µg
Human Interferon-alpha 2a	90158-A	20 µg
Human Interferon-alpha 2b	90159-A	20 µg
STAT3, GST-tag	75003	20 µg
ISRE Reporter – HEK293 Recombinant Cell Line	60510	2 vials
ISRE Reporter Kit (JAK/STAT Signaling Pathway)	60613	500 rxns.
Jak1, GST-tag	40449	10 µg
Jak2 (JH1 domain), His-tag	40450	10 µg
Jak2 (JH1, JH2 domain), His-tag	40451	10 µg

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References

1. Decker T, Kovarik P, Meinke A. (1997) GAS elements: a few nucleotides with a majorimpact on cytokine-induced gene expression. *J Interferon Cytokine Res.* **17(3)**: 121-34.
2. Darnell J, Kerr IM, Stark GR. (1994) Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science* **264**: 1415-1421.

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