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Data Sheet

JAK/STAT Signaling Pathway ISRE Reporter – HEK293 Cell Line Catalog #: 60510

Product Description

The *ISRE Reporter – HEK293 Cell Line* is designed for monitoring the activity of the JAK/STAT signaling pathway. The JAK (Janus kinase) /STAT (Signal Transducer and Activator of Transcription) pathway is activated by various cytokines and growth factors and plays a critical role in cell growth, hematopoiesis, and immune response. In mammals, there are four JAKs (JAK1, JAK2, JAK3 and TYK2) and seven STAT proteins.

Binding of Interferon alpha ($\text{IFN}\alpha$) to its receptor leads to the activation of JAK1 and TYK2, which in turn phosphorylate and activate STAT1 and STAT2. The phosphorylated STAT1 and 2 form a heterodimer and bind to IRF9/p48, forming a protein complex known as ISGF3. This complex translocates to the nucleus and binds to the ISRE (Interferon Stimulated Response Element) in the promoter region thereby promoting transcription of interferon-inducible genes.

The *ISRE Reporter – HEK293 Cell Line* contains the firefly luciferase gene under the control of ISRE stably integrated into HEK293 cells. This cell line is validated for the response to stimulation with interferon Alpha A and to treatment with JAK inhibitor.

Application

- Monitor $\text{IFN}\alpha$ -induced activity and the JAK/STAT pathway activity.
- Screen for activators or inhibitors of the JAK/STAT pathway.

Format

Each vial contains $\sim 1.5 \times 10^6$ cells in 1 ml of 10% DMSO.

Storage

Immediately upon receipt, store in liquid nitrogen.

General Culture Conditions

Thaw Medium 1 (BPS Cat. #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 1B (BPS Cat. #79531): Thaw Medium 1 (BPS Cat. #60187) plus 400 $\mu\text{g}/\text{ml}$ of Geneticin (Life Technologies #11811031).

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Cells should be grown at 37°C with 5% CO₂ using Growth Medium 1B (Thaw Medium 1 plus Geneticin).

To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 1 (**no Geneticin**) spin down cells, resuspend cells in pre-warmed Thaw Medium 1 (**no Geneticin**), transfer resuspended cells to a T25 flask and culture in a CO₂ incubator at 37°C. At first passage, switch to Growth Medium 1B (**contains Geneticin**). Cells should be split before they reach complete confluence.

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from the culture vessel with 0.05% Trypsin/EDTA. Add Growth Medium 1B and transfer to a tube, spin down the cells, then resuspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 weekly or twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with Trypsin/EDTA. Add Growth Medium 1B and transfer to a tube, spin down cells, and resuspend in freezing medium (10% DMSO + 90% FBS). Place at -80°C overnight and place in liquid nitrogen the next day. Alternatively, vials may be placed directly in liquid nitrogen.

Functional Validation and 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 volumes should be scaled appropriately.

Materials Required but Not Supplied

- Human Interferon Alpha A (IFN α) (R&D Systems # 11100-1)
- JAK Inhibitor I (Pyridone 6) (EMD Millipore # 420099). Prepare stock solution in DMSO.
- Assay Medium: Thaw Medium 1 (BPS Cat. #60187)
- Growth Medium 1B (BPS Cat. #79531)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Cat. #60690)
- Luminometer

Mycoplasma testing

The cell line has been screened using the PCR-based VenorGeM® Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of Mycoplasma species.

A. Dose response of ISRE Reporter – HEK293 cells to IFN α

1. Harvest ISRE Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of 30,000 cells per well into a white clear-bottom 96-well microplate in 45 μ l of Thaw Medium 1.

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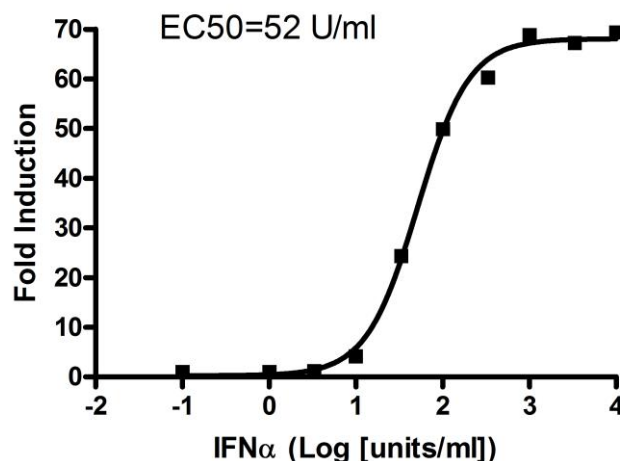
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2. Incubate cells at 37°C in a CO₂ incubator overnight.
3. The next day, prepare threefold serial dilutions of IFN α in assay medium and add 5 μ l of each dilution to stimulated wells.
Add 5 μ l of assay medium without IFN α to the unstimulated control wells.
Add 50 μ l of assay medium without IFN α to cell-free control wells (for determining background luminescence).
Set up each treatment in at least triplicate.
4. Incubate the plate at 37°C in a CO₂ incubator for 6 hours.
5. Perform luciferase assay using the ONE-Step™ Luciferase Assay System: add 100 μ l of ONE-Step™ Luciferase working solution mix per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.
If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.
6. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
The fold induction of ISRE luciferase reporter expression = background-subtracted luminescence of IFN α -stimulated well / average background-subtracted luminescence of unstimulated control wells

Figure 1. Dose Response of ISRE Reporter – HEK293 Cells to IFN α . The results are shown as fold induction of ISRE luciferase reporter expression.



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B. Inhibition of IFN α -induced reporter activity by JAK inhibitor in ISRE Reporter – HEK293 cells

1. Harvest ISRE Reporter – HEK293 cells from culture in Growth Medium 1B and seed cells at a density of 30,000 cells per well into a white clear-bottom 96-well microplate in 45 μ l of Thaw Medium 1.
2. Incubate cells at 37°C in a CO₂ incubator overnight.
3. The next day, prepare threefold serial dilutions of JAK Inhibitor I in assay medium and add 5 μ l of diluted inhibitor to the wells. The final concentration of DMSO in the wells can be up to 0.5%.
 Add 5 μ l of assay medium with same concentration of DMSO without inhibitor to inhibitor control wells.
 Add 50 μ l of assay medium with DMSO to cell-free control wells (for determining background luminescence).
4. Incubate the plate at 37°C in a CO₂ incubator for 1 hour.
5. Add 5 μ l of diluted IFN α in assay medium to stimulated wells (final [IFN α] = 1000 U/ml).
 Add 5 μ l of assay medium to the unstimulated control wells (cells without inhibitor and IFN α treatment for determining the basal activity).
 Add 5 μ l of assay medium to cell-free control wells.
 Set up each treatment in at least triplicate.

Treatment Reference Guide

	Stimulated Wells		Unstimulated Control Wells	Cell-free Control Wells
	With inhibitor	Without inhibitor (control well)		
Step 3	5 μ l diluted inhibitor in assay medium	5 μ l assay medium with DMSO only	5 μ l assay medium with DMSO only	50 μ l assay medium with DMSO only
Step 5	5 μ l IFN α in assay medium (final [IFN α] = 1000 U/ml)	5 μ l IFN α in assay medium (final [IFN α] = 1000 U/ml)	5 μ l assay medium	5 μ l assay medium

6. Incubate the plate at 37°C in a CO₂ incubator for 6 hours.
7. Perform luciferase assay using ONE-Step™ Luciferase Assay System: Add 100 μ l of ONE-Step™ Luciferase assay working solution per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.

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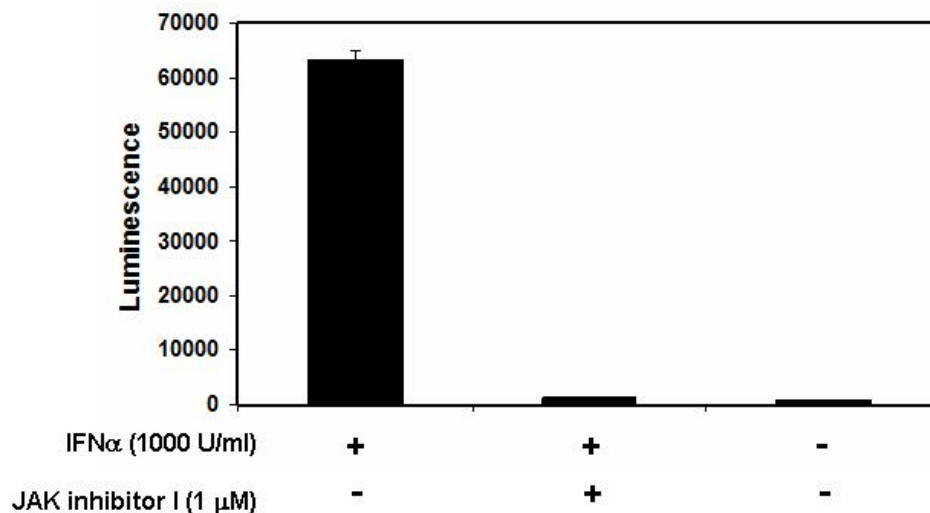
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If using other luciferase reagents from other vendors follow the manufacturer's assay protocol.

8. Data Analysis: Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Figure 2. Inhibition of IFN α -induced Reporter Activity by JAK Inhibitor I in ISRE Reporter – HEK293 Cells

2A. JAK Inhibitor I blocked IFN α -induced ISRE reporter activity.

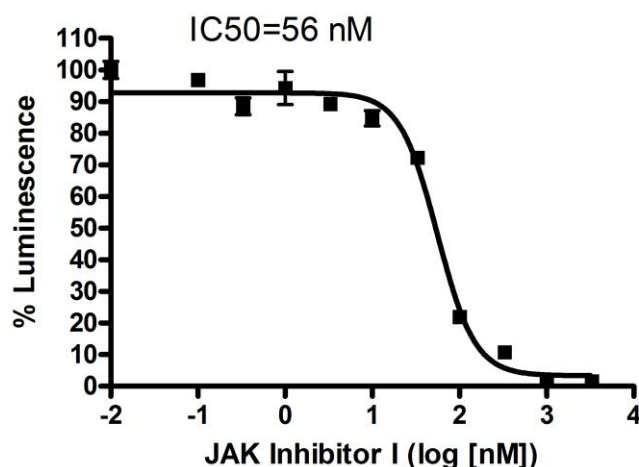


2B. JAK Inhibitor I inhibition dose response curve. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with IFN α in the absence of JAK Inhibitor I is set at 100%. The IC₅₀ of JAK Inhibitor I is ~ 56 nM

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1. Hebenstreit D et al. (2005) JAK/STAT-dependent gene regulation by cytokines. *Drug News Perspect* **18** (4): 243–249.
2. Pedranzini L et al. (2006) Pyridone 6, a pan-Janus-activated kinase inhibitor, induces growth inhibition of multiple myeloma cells. *Cancer Res.* **66** (19):9714-9721.

Related Products

Product Name	Catalog #	Size
JAK1 Recombinant Enzyme	40449	10 µg
JAK2 Recombinant Enzyme, JH1	40450	10 µg
JAK2 Recombinant Enzyme, JH1, JH2	40449	10 µg
JAK3 Recombinant Enzyme	40452	10 µg
IFN-alpha (2a) Recombinant	90158B	100 µg
IFN-alpha (2b) Recombinant	90159B	100 µg
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-1	100 ml

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