

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 (IFN α) 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 IFN α -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.

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: cell growth medium without Geneticin.

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- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- Luciferase reagents for measuring firefly luciferase activity (We use ONE-Glo™ luciferase assay system, Promega # E6110. Other luciferase assay systems are also suitable).
- Luminometer

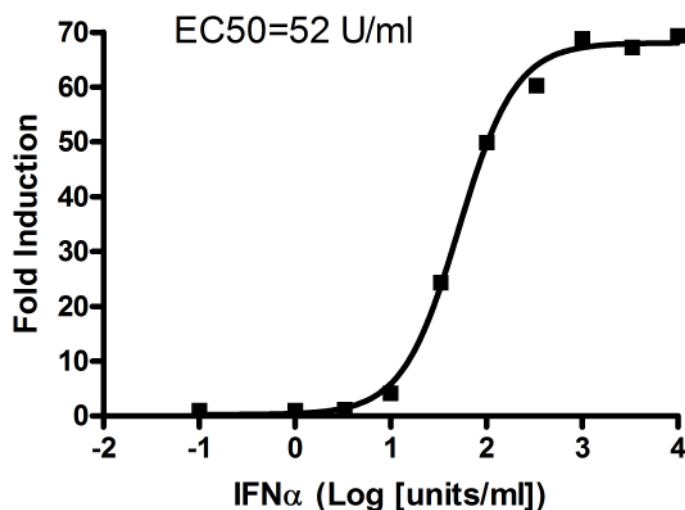
A. Dose response of ISRE Reporter – HEK293 cells to IFN α

1. Harvest ISRE Reporter – HEK293 cells from culture in growth medium 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 growth medium without Geneticin.
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-Glo luciferase assay system: add 50 μ l of One-Glo Luciferase reagent 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.

The EC₅₀ of IFN α is ~ 52 units/ml



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 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 growth medium without Geneticin.
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.

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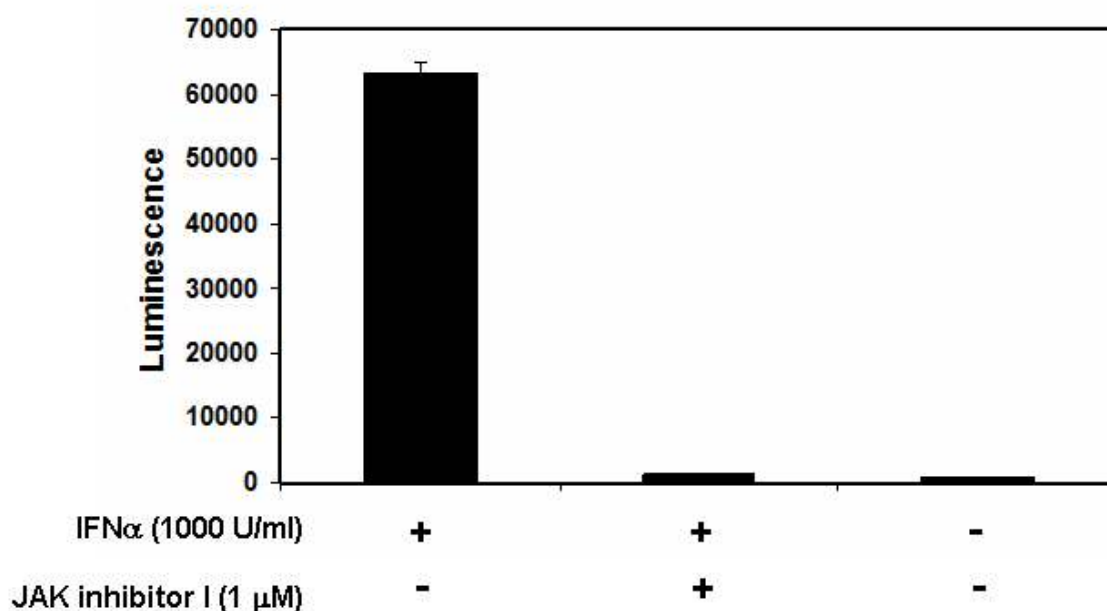
Treatment Reference Guide

	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

- Incubate the plate at 37°C in a CO₂ incubator for 6 hours.
- Perform luciferase assay using the ONE-Glo luciferase assay system: Add 50 μ l of One-Glo Luciferase reagent 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.
- 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

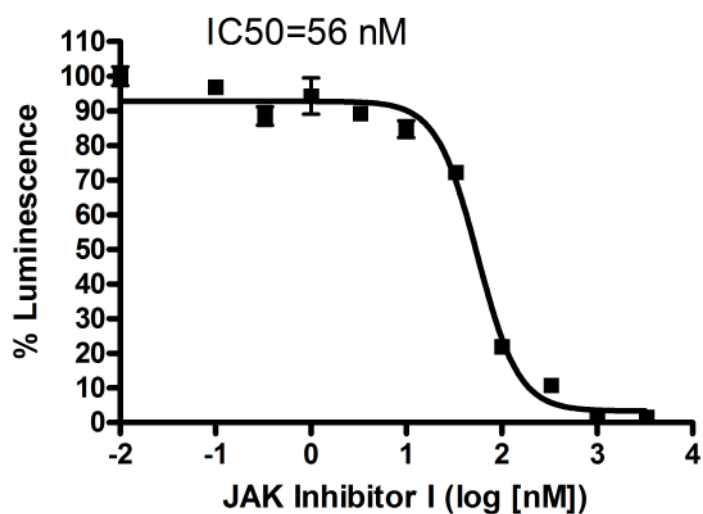
2A. JAK Inhibitor I blocked IFN α -induced ISRE reporter activity.



2B. JAK Inhibitor I inhibition dose response curve

The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with IFNα in the absence of JAK Inhibitor I is set at 100%.

The IC₅₀ of JAK Inhibitor I is ~ 56 nM



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.

Culture conditions

Cells should be grown at 37°C with 5% CO₂ using MEM medium (Hyclone #SH30024.01) supplemented with 10% FBS (Life Technologies #26140-079), 1% non-essential amino acids (Hyclone #SH30238.01), 1 mM Na-pyruvate (Hyclone #SH30239.01), 1% Penicillin/Streptomycin (Hyclone SV30010.01), and 400 µg/ml of Geneticin (Life Technologies #11811031). It may be necessary to adjust the percentage of CO₂ in the incubator depending on the NaHCO₃ level in the basal medium.

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 growth medium without Geneticin, spin down cells, resuspend cells in pre-warmed growth medium without Geneticin, transfer resuspended cells to a T25 flask and culture in a CO₂ incubator at 37°C. At first passage, switch to growth medium containing 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 complete growth medium 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.

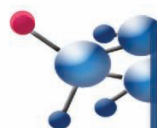
References

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

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
JAK/STAT Signaling Pathway GAS Reporter – HEK293 Cell Line <i>Coming Soon!</i>	60651	1 Vial
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

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