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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 (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.

General Culture Conditions

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 1B (BPS Bioscience #79531): Thaw Medium 1 (BPS Bioscience #60187) plus 400 μ g/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 Bioscience #60187)
- Growth Medium 1B (BPS Bioscience #79531)
- 96-well tissue culture plate or 96-well tissue culture-treated white clear-bottom assay plate
- ONE-Step™ Luciferase Assay System (BPS Bioscience #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.

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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.
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

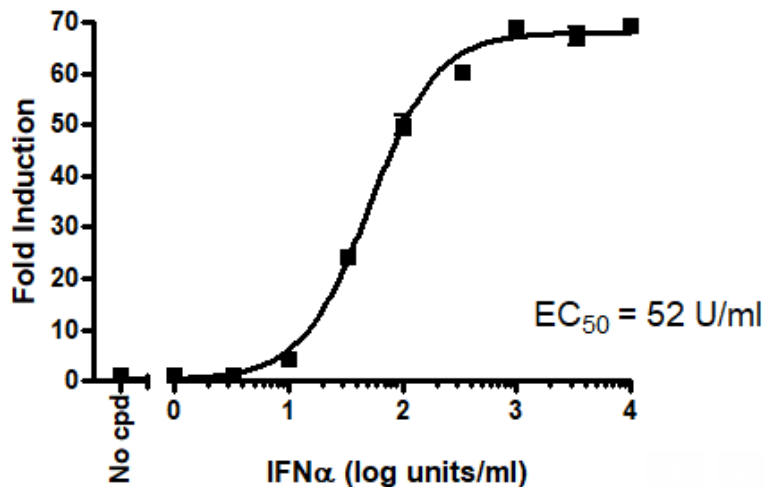
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Figure 1. Dose Response of ISRE Reporter – HEK293 Cells to IFN α . The results are shown as fold induction of ISRE luciferase reporter expression.



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.

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Set up each treatment in at least triplicate.

Treatment Reference Guide

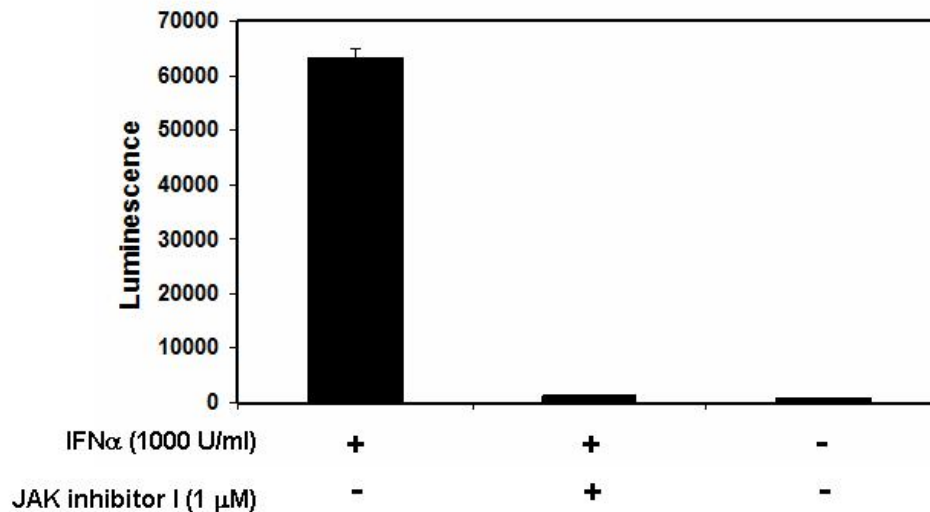
	Stimulated Wells		Unstimulated Control Wells	Cell-free Control Wells
	With inhibitor	Without inhibitor (control well)		
Step 3	5 μ l diluted inhibitor in assay medium	5 μ l assay medium with DMSO only	5 μ l assay medium with DMSO only	50 μ l assay medium with DMSO only
Step 5	5 μ l IFN α in assay medium (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

- Incubate the plate at 37°C in a CO₂ incubator for 6 hours.
- 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.
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.

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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.



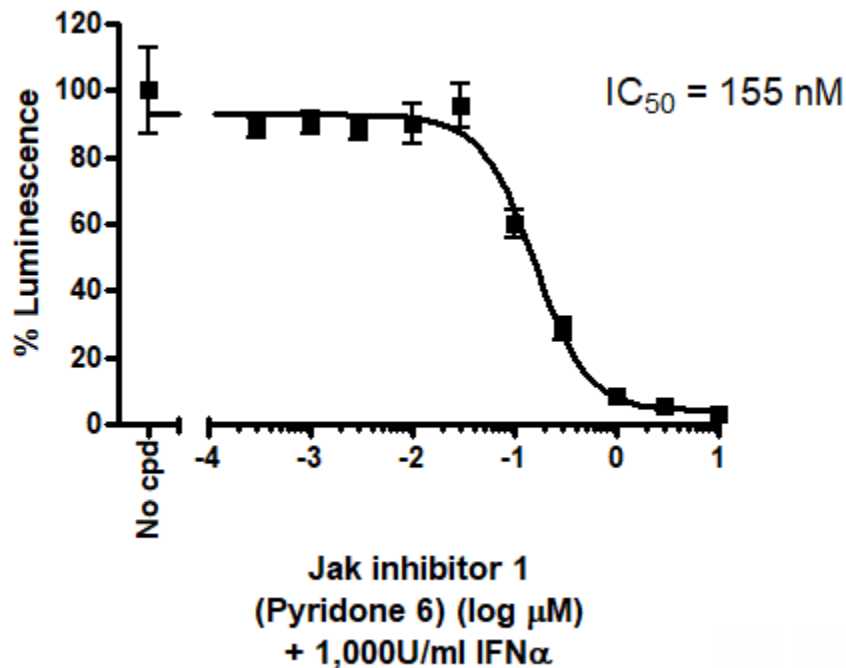
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2B. JAK Inhibitor I inhibition dose response curve. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with IFN α in the absence of JAK Inhibitor I is set at 100%.
 The IC₅₀ of JAK Inhibitor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



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.

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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
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