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Data Sheet

JAK/STAT Signaling Pathway
ISRE Reporter – HEK293 Cell Line
Catalog #: w70521

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 (IFNα) 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 IFNα-induced activity and the JAK/STAT pathway activity.
- Screen for activators or inhibitors of the JAK/STAT pathway.

Format
Each vial contains ~1.5 X 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 (IFNα) (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
- One-Step Luciferase Assay System (West Bioscience Cat. #w70701)
- 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.

Culture conditions
Cells should be grown at 37°C with 5% CO_2 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 CO_2 in the incubator depending on the $NaHCO_3$ 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 CO_2 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.

A. Dose response of ISRE Reporter – HEK293 cells to IFNα

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 µl of growth medium without Geneticin.

2. Incubate cells at 37°C in a CO_2 incubator overnight.

3. The next day, prepare threefold serial dilutions of IFNα in assay medium and add 5 µl of each dilution to stimulated wells.
   Add 5 µl of assay medium without IFNα to the unstimulated control wells.
   Add 50 µl of assay medium without IFNα 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 CO_2 incubator for 6 hours.

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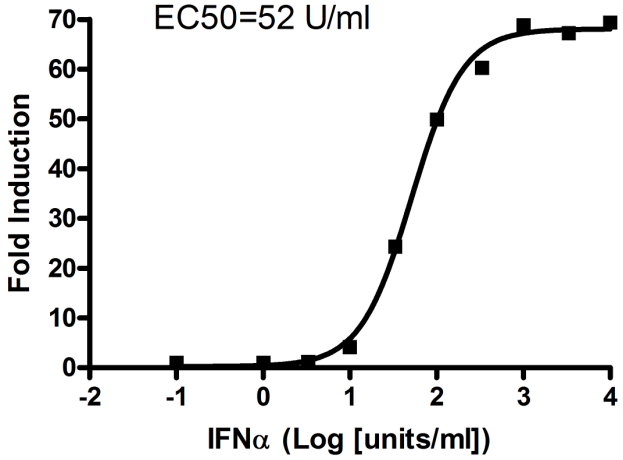
5. Perform luciferase assay using the One-Step Luciferase Assay System: add 100 µl of One-Step Luciferase working solution mix per well and rock at room temperature for ~15 minutes. Measure luminescence using a luminometer.
   If using other luciferase reagents from other vendors, follow the manufacturer's 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 IFNα-stimulated well / average background-subtracted luminescence of unstimulated control wells

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



B. Inhibition of IFNα-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 µl of growth medium without Geneticin.

2. Incubate cells at 37°C in a CO_2 incubator overnight.

3. The next day, prepare threefold serial dilutions of JAK Inhibitor I in assay medium and add 5 µl of diluted inhibitor to the wells. The final concentration of DMSO in the wells can be up to 0.5%.

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Add 5 µl of assay medium with same concentration of DMSO without inhibitor to inhibitor control wells.
Add 50 µl of assay medium with DMSO to cell-free control wells (for determining background luminescence).

4. Incubate the plate at 37°C in a CO_2 incubator for 1 hour.

5. Add 5 µl of diluted IFNα in assay medium to stimulated wells (final [IFNα] = 1000 U/ml).
Add 5 µl of assay medium to the unstimulated control wells (cells without inhibitor and IFNα treatment for determining the basal activity).
Add 5 µl 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 µl diluted inhibitor in assay medium	5 µl assay medium with DMSO only	5 µl assay medium with DMSO only	50 µl assay medium with DMSO only
Step 5	5 µl IFNα in assay medium (final [IFNα] = 1000 U/ml)	5 µl IFNα in assay medium (final [IFNα] = 1000 U/ml)	5 µl assay medium	5 µl assay medium

6. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.

7. Perform luciferase assay using One-Step Luciferase Assay System: Add 100 µl 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.

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.

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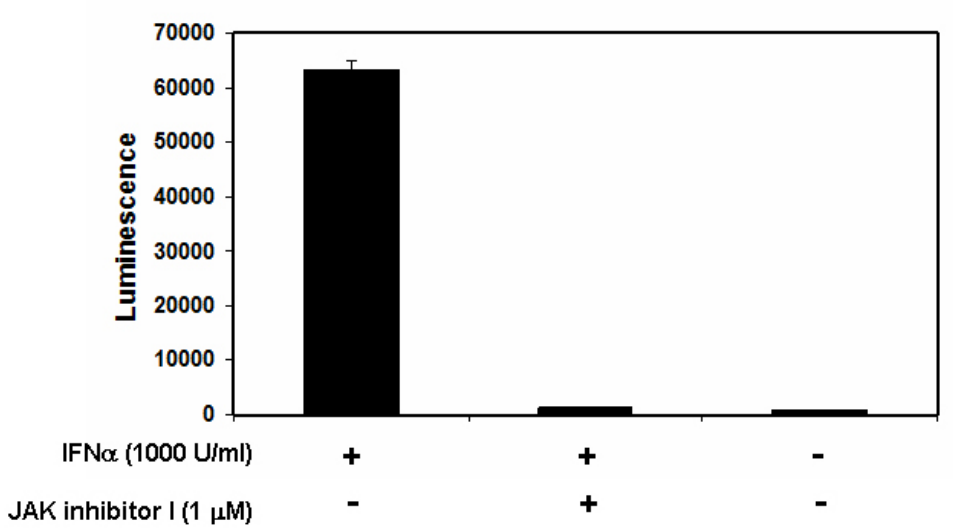
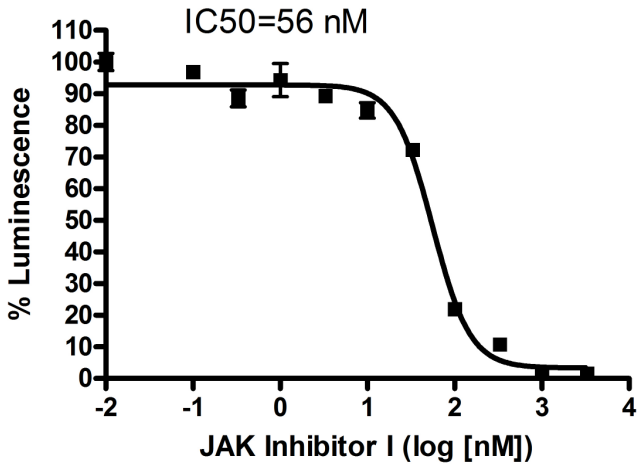
Figure 2. Inhibition of IFNα-induced Reporter Activity by JAK Inhibitor I in ISRE Reporter – HEK293 Cells

2A. JAK Inhibitor I blocked IFNα-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



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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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