Description

The ISRE Reporter – HEK293 Cell Line is designed to monitor 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 phosphorylates and activates 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 and to treatment with JAK inhibitor Pyridone 6.

Application

- Monitor IFNα-induced activity and JAK/STAT pathway activity.
- Screen for compound effect on the JAK/STAT pathway.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ~2 X 10^6 cells in 1 ml of 90% FBS, 10% DMSO

Host Cell

HEK293, epithelial-like cells, adherent

Mycoplasma Testing

The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

Materials Required for Cellular Assay

Name	Ordering Information
Human Interferon Alpha A (IFNα)	R&D Systems # 11100-1/PBL Assay Service # 11100-1
JAK Inhibitor I (Pyridone 6): Prepare stock solution in DMSO.	EMD Millipore # 420099
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	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations

For best results, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2. BPS cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium (Thermo Fisher, #11095098) supplemented with 10% FBS (Thermo Fisher, #26140079), 1% non-essential amino acids (Corning, #25-025-CI), 1 mM Na pyruvate (Corning, #25-000-CI), 1% Penicillin/Streptomycin (Thermo Fisher, #15140163)

Growth Medium 1B (BPS Bioscience #79531):
Thaw Medium 1 (BPS Bioscience #60187) plus 400 µg/ml of Geneticin (Life Technologies #11811031).

Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)

Cell Culture Protocol

Cell Thawing

1. 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 the cells down, resuspend cells in pre-warmed Thaw Medium 1 (**no Geneticin**), transfer resuspended cells to a T25 flask and culture in a CO_2 incubator at 37°C.
2. At first passage, switch to Growth Medium 1B (**contains Geneticin**). Cells should be split before they reach complete confluence.

Cell Passage

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

Cell Freezing

1. To cryopreserve the cells, remove the medium, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA.
2. After detachment, add Growth Medium 1B and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (BPS Bioscience #79796 or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.
3. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight.
4. Transfer to liquid nitrogen the next day for storage.

Note: It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

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.

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

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 75 µl of Thaw Medium 1.
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 at 4x final concentration and add 25µl of each dilution to stimulated wells.
   Add 25 µl of assay medium without IFNα to the unstimulated control wells.
   Add 100 µl of assay medium without IFNα to cell-free control wells (for determining background luminescence).
   Set up each treatment in triplicate.
4. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.
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

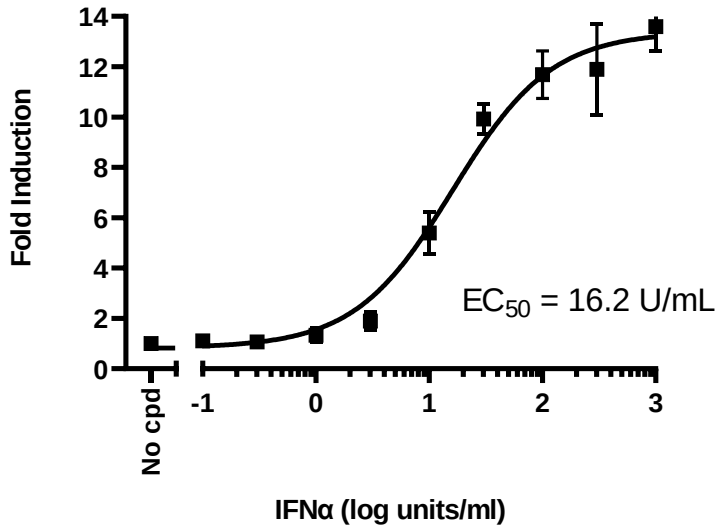

Figure 1. Dose Response of ISRE Reporter – HEK293 Cells to IFNα. The results are shown as fold induction of ISRE luciferase reporter expression.

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 1B and seed cells at a density of 30,000 cells per well into a white clear-bottom 96-well microplate in 100 µl of Thaw Medium 1.
2. Incubate cells at 37°C in a CO_2 incubator overnight.
3. The next day, remove the medium and feed cells with 40 µl of assay medium. Prepare threefold serial dilutions of JAK Inhibitor I (Pyridone 6) in assay medium at 2x the final concentration and add 50 µl of diluted inhibitor to the wells. The final concentration of DMSO in the wells can be up to 0.5%.
   Add 50 µl of assay medium containing the same concentration of DMSO but without inhibitor to the "no inhibitor" control wells.
   Add 100 µl of assay medium 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 10 µl of diluted IFNα in assay medium to stimulated wells (final [IFNα] = 1000 U/ml).
   Add 10 µl of assay medium to the unstimulated control wells (cells without inhibitor and without IFNα treatment for determining the basal activity).

Treatment Reference Guide

	Stimulated Wells		Unstimulated Control Wells	Cell-free Control Wells
	With Inhibitor	Without Inhibitor (control well)		
Step 3	50 µl diluted inhibitor in assay medium	50 µl assay medium with DMSO only	50 µl assay medium with DMSO only	100 µl assay medium
Step 5	10 µl IFNα in assay medium (final [IFNα] = 1000 U/ml)	10 µl IFNα in assay medium (final [IFNα] = 1000 U/ml)	10 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. The percent luminescence of ISRE luciferase reporter expression = background- subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells x 100%

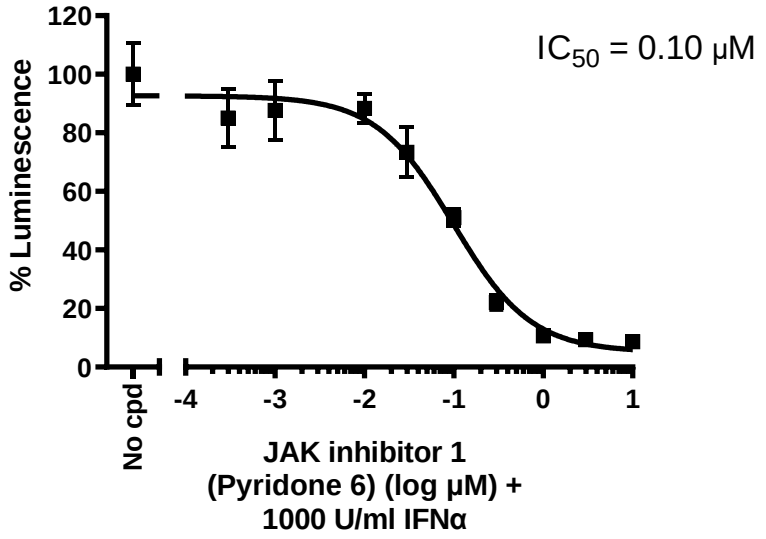

Figure 2. Inhibition of IFNα-induced Reporter Activity by JAK Inhibitor I in ISRE Reporter – HEK293 Cells. The results are shown as percentage of luminescence. The background-subtracted luminescence of cells stimulated with IFNα in the absence of JAK Inhibitor I is set at 100%.
The IC50 of JAK Inhibitor I is 0.10 µM

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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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
JAK1, GST-tag	40449	10 µg
JAK2 (JH1 domain), His-tag	40450	10 µg
JAK2 (JH1, JH2 domain), His-GST-tags	40451	20 µg
JAK3, His-tag	40452	20 µg
ONE-Step™ Luciferase Assay System	60690	Multiple sizes
Thaw Medium 1	60187	100 ml/500 ml
Growth Medium 1B	79531	500 ml