6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

ISRE Reporter Kit
JAK/STAT Signaling Pathway
Catalog #: 60613

Background

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. Interferon alpha (IFNα) is a Type I interferon. Binding of 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 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.

Description

The ISRE Reporter kit is designed for monitoring the activity of Type I interferon-induced JAK/STAT signaling pathway in the cultured cells. The kit contains transfection-ready ISRE luciferase reporter vector, which is a JAK/STATpathway-responsive reporter. This reporter contains a firefly luciferase gene under the control of multimerized ISRE responsive element located upstream of a minimal promoter. The ISRE reporter is premixed with constitutively-expressing *Renilla* luciferase vector, which serves as an internal control for transfection efficiency.

The kit also includes a non-inducible firefly luciferase vector premixed with constitutively-expressing *Renilla* luciferase vector as a negative control. The non-inducible luciferase vector contains a firefly luciferase gene under the control of a minimal promoter, without any additional response elements. The negative control is critical to determining pathway specific effects and background luciferase activity.

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Applications

- Monitor IFNα-induced JAK/STAT pathway activity.
- Screen activators or inhibitors of the JAK/STAT signaling pathway.
- Study effects of RNAi or gene overexpression on the activity of the JAK/STAT pathway.

Components

Component	Specification	Amount	Storage
Reporter (Component A)	ISRE luciferase reporter vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/ µl)	-20°C
Negative Control Reporter (Component B)	Non-inducible luciferase vector + constitutively expressing *Renilla* luciferase vector	500 µl (60 ng DNA/ µl)	-20°C

These vectors are ready-to-use for transient transfection. They are NOT SUITABLE for transformation and amplification in bacteria.

Materials Required but Not Supplied

- Mammalian cell line and appropriate cell culture medium
- 96-well tissue culture plate or 96-well tissue culture treated white clear-bottom assay plate (Corning # 3610)
- Transfection reagent for mammalian cell line [We use Lipofectamine™ 2000 (Invitrogen # 11668027). However, other transfection reagents work equally well.
- Opti-MEM I Reduced Serum Medium (Invitrogen #31985-062)
- Dual luciferase assay system:
  Dual-Glo® Luciferase Assay System (Promega #E2920): This system assays cells directly in growth medium. It can be used with any luminometer. Automated injectors are not required.

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

OR
Dual-Luciferase® Reporter Assay System (Promega #E1910): This system requires a cell lysis step. It is ideal for use with a luminometer with automated injectors.

- Luminometer

Generalized Transfection and Assay Protocols

The following procedure is designed to transfect the reporter into HEK293 cells using Lipofectamine 2000 in a 96-well format. To transfect cells in different tissue culture formats, adjust the amounts of reagents and cell number in proportion to the relative surface area. If using a transfection reagent other than Lipofectamine 2000, follow the manufacturer's transfection protocol. Transfection condition should be optimized according to the cell type and study requirements.

All amounts and volumes in the following setup are given on a per well basis.

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. The next day, for each well, prepare complexes as follows:

a. Dilute DNA mixtures in 15 µl of Opti-MEM I medium (antibiotic-free). Mix gently. Depending upon the experimental design, the DNA mixtures may be any of following combinations:

- **1 µl of Reporter** (component A); in this experiment, the control transfection is **1 µl of Negative Control Reporter** (component B).
- **1 µl of Reporter** (component A) + experimental vector expressing gene of interest; in this experiment, the control transfection is: **1 µl of Reporte**r (component A) + negative control expression vector, **1 µl of Negative Control Reporter** (component B) + experimental vector expressing gene of interest, and **1 µl of Negative Control Reporter** (component B) + negative control expression vector.
- **1 µl of Reporter** (component A) + specific siRNA; in this experiment, the control transfection is: **1 µl of Reporte**r (component A) + negative control siRNA, **1 µl of Negative Control Reporter** (component B) + specific siRNA, and **1 µl of Negative Control Reporter** (component B) + negative control siRNA.

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Note: we recommend setting up at least triplicate assays for each condition, and preparing transfection cocktail for multiple wells.

b. Mix Lipofectamine 2000 gently before use, then dilute 0.35 µl of Lipofectamine 2000 in 15 µl of Opti-MEM I medium (antibiotic-free). Incubate for 5 minutes at room temperature.
Note: Prepare this dilution cocktail in volumes sufficient for the whole experiment.

c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine 2000. Mix gently and incubate for 25 minutes at room temperature.

3. Add the 30 µl of complexes to each well containing cells and medium. Mix gently by tapping the plate.

4. Incubate cells at 37°C in a CO_2 incubator. After ~24 hours of transfection, treat cells with test activators/inhibitors for additional 6 to 24 hours. Perform the dual luciferase assay following the manufacturer's protocol.

Sample protocol to determine the dose response of HEK293 cells transfected with ISRE reporter to IFNα

Additional materials required in this experiment setup

- Human Interferon Alpha A (IFNα) (R&D Systems # 11100-1)
- HEK293 growth medium: MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) + 10% FBS + 1% non-essential amino acids + 1 mM Na pyruvate + 1% Pen/Strep
- Assay medium: same as HEK293 growth medium
- Dual-Glo® Luciferase Assay System (Promega #E2920)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into a white clear-bottom 96-well plate in 100 µl of growth medium. Incubate cells at 37°C in a CO_2 incubator for overnight.

2. Next day, transfect 1 µl of ISRE luciferase reporter (component A) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, dilute IFNα in assay medium and replace cell medium in the stimulated wells with 50 µl of diluted IFNα.

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Change medium in the unstimulated control wells to 50μl of assay medium; add 50 μl of assay medium to cell-free control wells (for determining background luminescence).
Set up each treatment in at least triplicate.

4. Incubate cells at 37° in a CO_2 incubator for ~ 6 hours.

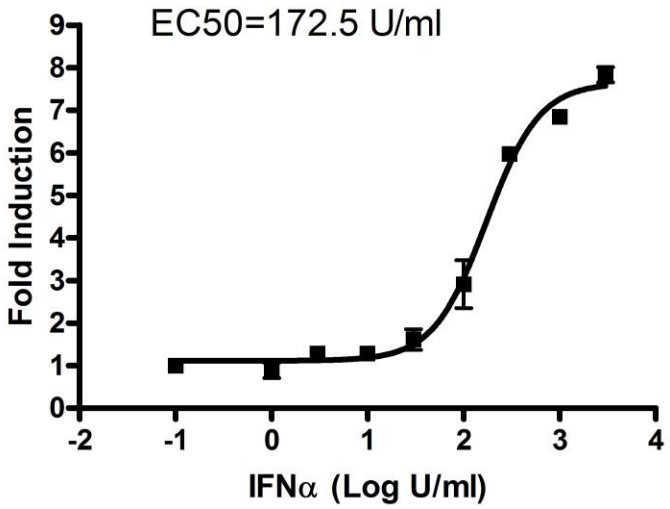
5. Perform the dual luciferase assay using Dual-Glo® Luciferase Assay System: Add 50 μl of Luciferase reagent per well, rock at room temperature for ~15 minutes, and measure firefly luminescence using a luminometer. Add 50 μl of Stop & Glo® reagent per well, rock at room temperature for ~15 minutes, and measure *Renilla* luminescence.

7. To obtain the normalized luciferase activity for ISRE reporter, subtract background luminescence, then calculate the ratio of firefly luminescence from the ISRE reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

Figure 1. Dose response of ISRE reporter activity to IFNα in HEK293
The results are shown as fold induction of normalized ISRE luciferase reporter activity. Fold induction was determined by comparing values against the mean value for control cells without IFNα treatment.

The EC50 of IFNα is ~ 172.5 units/ml

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com



Sample protocol to determine the effects of JAK inhibitor on IFNα-induced ISRE reporter activity in HEK293 cells

Additional materials required in this experiment setup

- JAK Inhibitor I (Pyridone 6) (Calbiochem # 420099): inhibitor of JAKs. Prepare stock solution in DMSO.
- Human Interferon Alpha A (IFNα) (R&D Systems # 11100-1)
- HEK293 growth medium: MEM/EBSS (with L-glutamine) (Hyclone #SH30024.01) + 10% FBS + 1% non-essential amino acids + 1 mM Na pyruvate + 1% Pen/Strep
- Assay medium: same as HEK293 growth medium
- Dual-Glo® Luciferase Assay System (Promega #E2920)

1. One day before transfection, seed HEK293 cells at a density of 30,000 cells per well into a white clear-bottom 96-well plate in 100 μl of growth medium. Incubate cells at 37°C in a CO_2 incubator for overnight.

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

2. The next day, transfect 1 μl of ISRE luciferase reporter (component A) into cells following the procedure in **Generalized Transfection and Assay Protocols.**

3. After ~24 hours of transfection, prepare threefold serial dilution of JAK Inhibitor I assay medium. Replace the cell medium with 50 μl of diluted JAK inhibitor I. The final concentration of DMSO in the wells is 0.1%.
Change medium to 50 μl of 0.1% DMSO assay medium to wells without inhibitor and cell-free control wells (for determining background luminescence).

Incubate cells at 37°C in a CO_2 incubator for ~ 1 hours

4. Add 5 μl of diluted IFNα in assay medium to stimulated wells (final IFNα concentration = 1000 U/ml).
Add 5 μl of assay medium to the unstimulated control wells (cells without inhibitor and IFNα treatment) to determine the basal activity.
Add 5 μl of assay medium to cell-free control wells.
Set up each treatment in at least triplicate.

5. Incubate at 37°C in a CO_2 incubator for ~6 hours.

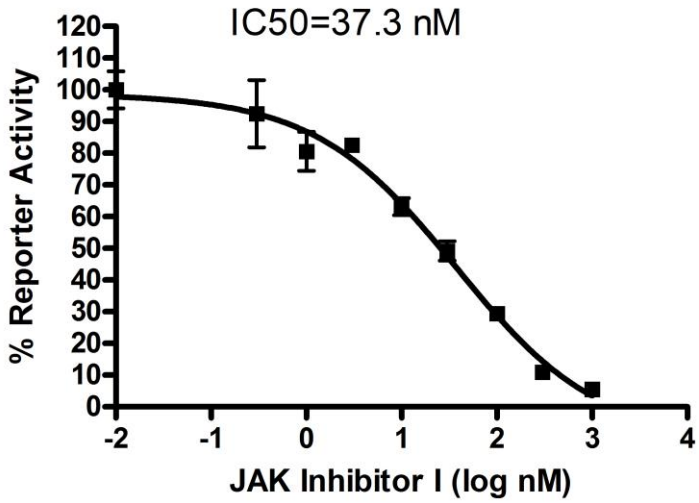
6. Perform the dual luciferase assay using Dual-Glo® Luciferase Assay System: Add 55 μl of Luciferase reagent per well, rock at room temperature for ~15 minutes, and measure firefly luminescence using a luminometer. Add 55 μl of Stop & Glo® reagent per well, rock at room temperature for ~15 minutes, and measure *Renilla* luminescence.

7. To obtain the normalized luciferase activity of ISRE reporter, subtract background luminescence then calculate the ratio of firefly luminescence from the ISRE reporter to *Renilla* luminescence from the control *Renilla* luciferase vector.

Figure 2. JAK Inhibitor I inhibition dose response curve
The results are shown as the percentage of ISRE reporter activity. The normalized luciferase activity for cells stimulated with IFNα in the absence of JAK inhibitor I was set at 100%.

The IC50 of JAK inhibitor I is ~ 37. 3 μM.

6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com



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

Product	**Catalog #**	**Size**
ISRE Reporter - HEK293 Cell Line	60510	2 vials
Jak2 (JH2 domain) Enzyme	40449	10 µg
Jak2 (JH2 domain) Enzyme	40450	10 µg
Jak2 (JH1, JH2 domain) Enzyme	40451	10 µg
Jak3 Enzyme	40452	10 µg