6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

ISRE Luciferase Reporter Lentivirus (JAK/STAT Signaling Pathway)

Catalog #: 79824

Description

The JAK/STAT 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. 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.

The ISRE Luciferase Reporter Lentivirus are replication incompetent, HIV-based, VSV-G pseudotyped lentiviral particles that are ready to be transduced into almost all types of mammalian cells, including primary and non-dividing cells. The particles contain a firefly luciferase gene driven by multimerized ISRE response element located upstream of the minimal TATA promoter (Figure 1). After transduction, the activity of Type I interferon-induced JAK/STAT signaling pathway in the target cells can be monitored by measuring the luciferase activity.

Application

- Screen for activators or inhibitors of Type I interferon induced JAK/STAT signaling pathway in the transduced target cells
- Generation of ISRE Luciferase Reporter stable cell line

Formulation

The lentiviruses were produced from HEK293T cells in the medium containing 90% DMEM + 10% FBS.

Titer

Two vials (500 µl x 2) of ISRE luciferase reporter lentivirus at a titer 5 x 10^6 TU/ml. The titer will vary with each lot; the exact value is provided with each shipment.

Storage

Lentiviruses are shipped with dry ice. For long term storage, it is recommended to store the virus at -80°C. Avoid repeated freeze-thaw cycles. Titers can drop significantly with each freeze-thaw cycle.

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Biosafety

The lentiviruses are produced with the third generation SIN (self-inactivation) lentivector which ensures self-inactivation of the lentiviral construct after transduction and integration into the genomic DNA of the target cells. None of the HIV genes (gag, pol, rev) will be expressed in the transduced cells, as they are expressed from packaging plasmids lacking the packing signal.

Although the pseudotyped lentiviruses are replication-incompetent, they require the use of a Biosafety Level 2 facility. BPS recommends following all local federal, state, and institutional regulations and using all appropriate safety precautions.

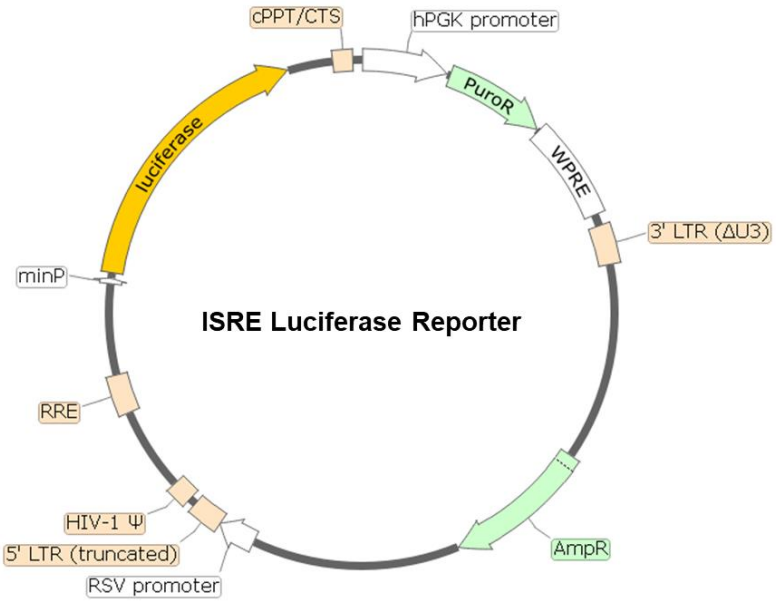

Figure 1. Schematic of the lenti-vector used to generate the ISRE luciferase reporter lentivirus

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Materials Required but Not Supplied

- Human Interferon Alpha A (IFNα) (R&D Systems # 11100-1)
- STING Ligands: 2,3 cGAMP (Invivogen # tlrl-nacga23), 3,3 cGAMP (Invivogen #tlrl-nacga)
- THP-1 cell (ATCC#TIB-202)
- THP-1 growth medium or use Thaw Medium 8 (BPS Bioscience #79652)
- Assay medium: RPMI 1640 medium (Life Technologies #A10491-01)
- Polybrene (Millipore, #TR-1003-G)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- One-Step luciferase assay system (BPS Bioscience, #60690)
- Luminometer

Assay Protocol

The following protocol is a general guideline for transducing THP-1 cells using ISRE luciferase reporter lentivirus. The optimal transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirement. In most cell types, the expression of the reporter gene can be measured approximately 72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the reporter gene with puromycin prior to carrying out the reporter assays.

1. Harvest the THP-1 cells by centrifugation and resuspend the cells in fresh THP-1 growth medium. Dilute the cells to 5 x 10^5 /ml in growth medium. Mix 800 µl of the THP-1 cells and 200 µl of ISRE luciferase reporter lentivirus in a 1.5-ml Eppendorf tube. Add polybrene to a final concentration of 8 µg/ml. Gently mix and incubate the virus with the THP-1 cells for 20 minutes at room temperature in the tissue culture hood.

2. Centrifuge the virus/cells mixture for 30 minutes at 800 x g at 32°C. Remove the virus containing medium and resuspend the cell pellet in 2 ml of fresh THP-1 growth medium. Transfer the cells into one well in a 6-well plate. Incubate the plate at 37°C with 5% CO_2 for 48-66 hours. The transduced THP-1 cells are ready for assay development.

3. Harvest the cells and resuspend the cells into 1.5 ml assay medium. Add 90 µl of the cells to each well of the 96-well plate. Dilute IFNα or STING ligand with assay medium. Add 10 µl of diluted IFNα or STING ligand to the stimulated wells. Add 10 µl of assay medium to the unstimulated control wells (for measuring the uninduced level of ISRE reporter activity).

4. Incubate the plate at 37°C with 5% CO_2 for 24 hours.

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5. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Important Notes:

1. To generate the ISRE luciferase reporter stable cell line, remove the growth medium 48 hours after transduction and replace it with fresh growth medium containing the appropriate amount of puromycin for antibiotic selection of transduced cells.

2. The following Lenti Reporter Controls are also available from BPS Bioscience to meet your experimental needs:

1) Negative Control Lentivirus (BPS Bioscience, #79578): Ready-to-transduce lentiviral particles expressing firefly luciferase under the control of a minimal promoter. The negative control is important to establish the specificity of any treatments and to determine the background reporter activity.

2) Renilla Luciferase (Rluc) Lentivirus (BPS Bioscience, #79565): Ready-to-transduce lentiviral particles expressing Renilla luciferase under the CMV promoter. The RLuc lentivirus can serve as an internal control to overcome sample-to-sample variability when performing dual-luciferase reporter assays.

3) Firefly Luciferase (Fluc) Lentivirus (BPS Bioscience, #79692-G, #79692-H, #79692-P): Ready-to-transduce lentiviral particles expressing firefly luciferase under the CMV promoter. The Fluc lentivirus can serve as a positive control for transduction optimization studies.

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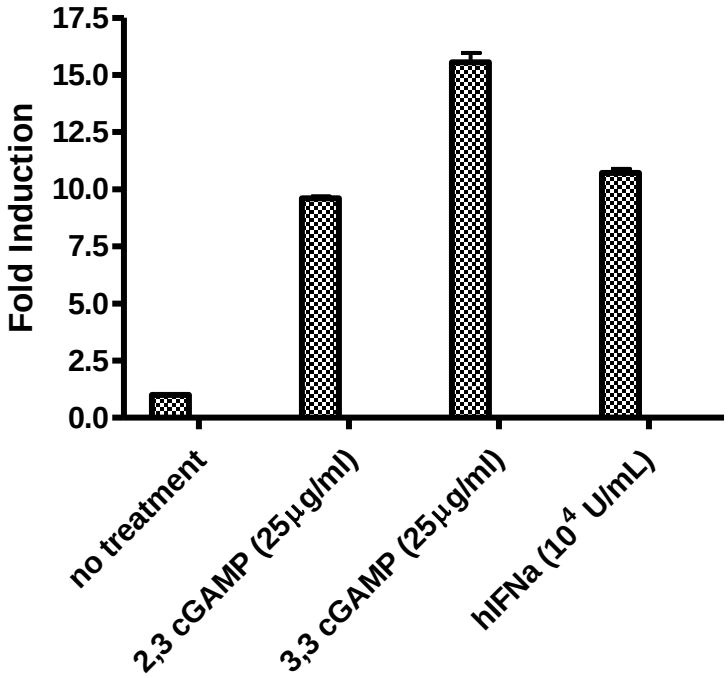

Figure 2. ISRE luciferase reporter activity stimulated by hIFNα and STING ligands in THP-1 cells. Appropriate 30,000 THP-1 cells/well were transduced with 100,000 TU/well ISRE luciferase reporter lentivirus. After 48 hours of transduction, growth medium was changed to assay medium, and the cells were treated with human IFNα (10^4 U/ml), STING ligand 2,3 cGAMP (25 µg/ml) or 3,3 cGAMP (25 µg/ml) for 24 hours. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without treatment.

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Related Products

Product	Cat. #	Size
NF-κB Luciferase Reporter Lentivirus	79564	500 µl x2
CRE Luciferase Reporter Lentivirus	79580	500 µl x2
NFAT Luciferase Reporter Lentivirus	79579	500 µl x2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x2
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x2
TCF/LEF Luciferase Reporter Lentivirus	79787	500 µl x2
IL-2 Promoter Luciferase Reporter Lentivirus	79825	500 µl x2
IL-8 Promoter Luciferase Reporter Lentivirus	79827	500 µl x2
Negative Control Lentivirus	79578	500 µl x2
Renilla Luciferase (Rluc) Lentivirus	79565	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (G418)	79692-G	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Hygromycin)	79692-H	500 µl x2
Firefly Luciferase (Fluc) Lentivirus (Puromycin)	79692-P	500 µl x2
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Dual Luciferase (Firefly-Renilla) Assay System	60683	10 ml

References

1. Hebenstreit, D., *et al.* (2005). JAK/STAT-dependent gene regulation by cytokines. *Drug News Perspect* **18 (4):** 243–249.