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## **Data Sheet**

### ***IRF Reporter (Luc) – THP-1 Cell line (cGAS-STING Signaling Pathway)*** **Catalog #: 79858**

#### **Product Description**

The Interferon Regulatory Factor (IRF) reporter (Luc)-THP-1 cell line is designed to study the activation and signaling of Cytosolic DNA Sensors (CDS) in human monocytic cell line THP-1. It contains a firefly luciferase gene driven by multimerized ISRE (Interferon Stimulated Response Element) located upstream of the minimal TATA promoter.

The cGAS-STING pathway acts to detect cytosolic DNA and induce an immune response. Briefly, upon binding DNA, the protein cGAS (cyclic GMP-AMP Synthase) triggers reaction of GTP and ATP to form cGAMP. cGAMP binds to STING (Stimulator of Interferon Genes) which triggers phosphorylation of IRF3 via TBK1. IRF3 can then bind to interferon-stimulated responsive elements (ISRE) in the nucleus and leads to IFN- $\alpha/\beta$  production. The IRF reporter (Luc)-THP-1 cell line is highly responsive to STING and CDS ligands.

#### **Application**

- Screen for cGAS and STING agonists

#### **Format**

Each vial contains  $\sim 5 \times 10^6$  cells in 1 ml of 10% DMSO

#### **Storage**

Immediately upon receipt, store in liquid nitrogen.

#### **Host Cell**

THP-1 human leukemia monocytic cell line.

#### **Mycoplasma Testing**

The cell line has been screened using the PCR-based Venor<sup>®</sup>GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

#### **General Culture Conditions**

**Thaw Medium 8 (BPS Bioscience, #79652):** RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% heat-inactivated FBS (Life Technologies #10082147), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

**Growth Medium 8A (BPS Bioscience, #79653):** Thaw Medium 8 (BPS Bioscience, #79652) plus 1  $\mu$ g/ml of Puromycin (Takara, #631306).

Cells should be grown at 37°C with 5% CO<sub>2</sub> using Growth Medium 8A.

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It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 8 (**no Puromycin**). Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 8 (**no Puromycin**). Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO<sub>2</sub> incubator. After 24 hours of culture, add an additional 1 – 2 ml of Thaw Medium 8 (**no Puromycin**). At first passage, switch to Growth Medium 8A (**contains Puromycin**). Cells should be split before they reach 2.0 x 10<sup>6</sup> cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.5 x 10<sup>6</sup> cells/ml. Do not allow the cell density to exceed 2.0 x 10<sup>6</sup> cells/ml.

### Assay Performance

The following assays are designed for 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

### Materials Required but Not Supplied

- IFNα (Invivogen, #rcyc-hifna10)
- IFNβ (R&D systems, #8499-IF-010)
- STING agonist: 2'3'-cGAMP (Invivogen, #tlrl-nacga23);
- STING agonist: 3'3'-cGAMP (Invivogen, #tlrl-nacga)
- Assay Medium: RPMI1640 medium (Life Technologies, #A10491-01)
- Growth Medium 8A (BPS Bioscience, #79653)
- 96-well tissue culture treated white clear-bottom assay plate (Corning, #3610)
- ONE-Step™ luciferase assay system (BPS Bioscience, #60690)
- Luminometer

### A. IFNα/β dose response

1. Harvest IRF reporter (Luc)-THP-1 cells from culture in Growth Medium 8A and resuspend the cells in assay medium. Seed cells at a density of ~40,000 cells per well into a white opaque 96-well microplate in 90 µl of assay medium. Leave a few wells empty for use as a control for background luminescence.
2. Prepare threefold serial dilutions of IFNα or IFNβ in assay medium. Add 10 µl of diluted IFNα or IFNβ to stimulated wells.
3. Add 10 µl of assay medium to the unstimulated control wells (for measuring uninduced level of IRF reporter activity).
4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
5. Incubate at 37°C with 5% CO<sub>2</sub> for 24 hours.

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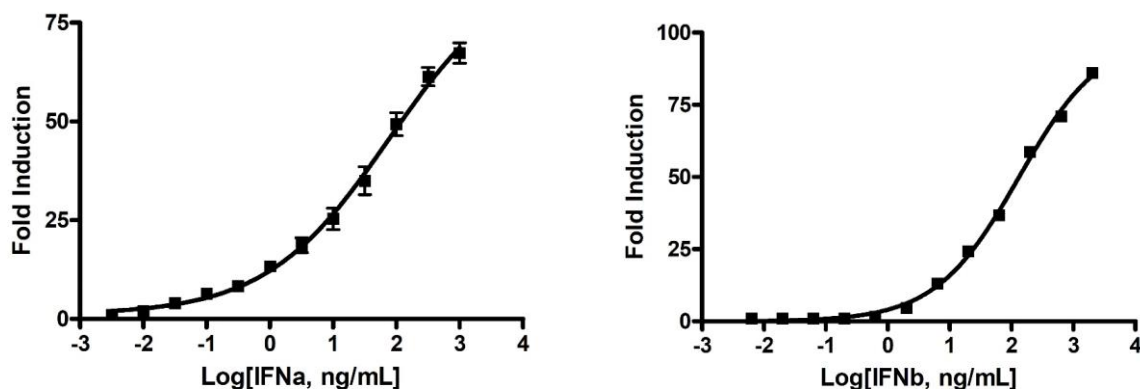
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6. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

**Figure 1. IFN $\alpha$ / $\beta$  dose response in IRF reporter (Luc)-THP-1 cells.** Cells were treated in assay medium with IFN $\alpha$  or IFN $\beta$  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.



## B. STING agonist dose response

1. Harvest IRF reporter (Luc)-THP-1 cells from culture in Growth Medium 8A and resuspend the cells in assay medium. Seed cells at a density of ~40,000 cells per well into a white opaque 96-well microplate in 90 µl of assay medium. Leave a few wells empty for use as a control for background luminescence.
2. Prepare threefold serial dilution of STING agonist in assay medium. Add 10 µl of diluted STING agonist to stimulated wells.
3. Add 10 µl of assay medium to the unstimulated control wells (for measuring uninduced level of IRF reporter activity).
4. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
5. Incubate at 37°C with 5% CO<sub>2</sub> for 24 hours.

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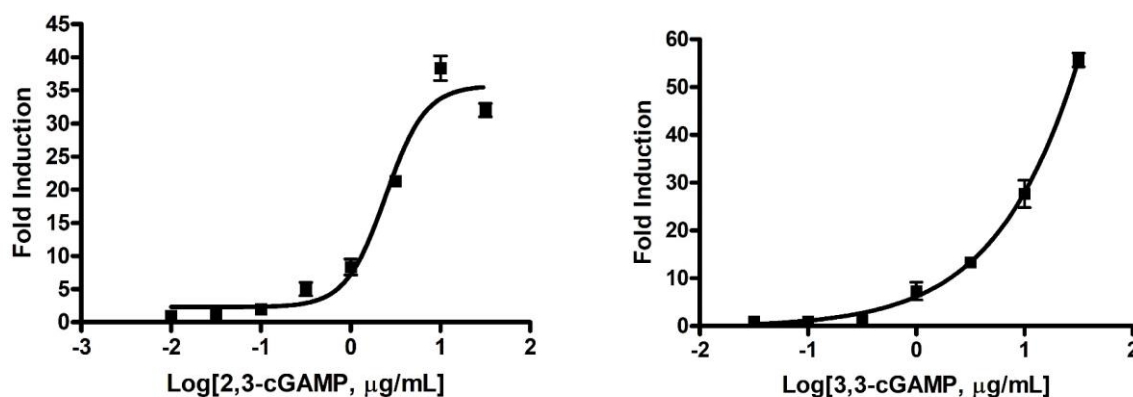
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6. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100  $\mu$ l of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

**Figure 2. STING agonist dose response in IRF reporter (Luc)-THP-1 cells.** Cells were treated with 2'3'-cGAMP or 3'3'-cGAMP in assay medium 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.



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## Related Products

<b><u>Product</u></b>	<b><u>Cat. #</u></b>	<b><u>Size</u></b>
NF- $\kappa$ B reporter (Luc) – THP-1 Cell line	79645	2 vials
ISRE Reporter – HEK293 Recombinant Cell Line	60510	2 vials
ISRE Reporter Kit (JAK/STAT Signaling Pathway)	60613	500 rxns.
ISRE Luciferase Reporter Lentivirus	79824	2 vials
ONE-Step™ Luciferase Assay System	60690-2	100 ml
Thaw Medium 8	79652	100 ml
Growth Medium 8A	79653	500 ml

## License Disclosure

Purchase of this cell line grants you with a 10-year license to use this cell line in your immediate laboratory, for research use only. This license does not permit you to share, distribute, sell, sublicense, or otherwise make the cell line available for use to other laboratories, departments, research institutions, hospitals, universities, or biotech companies. The license does not permit use of this cell line in humans or for therapeutic or drug use. The license does not permit modification of the cell line in any way. Inappropriate use or distribution of this cell line will result in revocation of the license and result in an immediate cease of sales and distribution of BPS products to your laboratory. BPS does not warrant the suitability of the cell line for any particular use, and does not accept any liability in connection with the handling or use of the cell line. Modifications of this cell line, transfer to another facility, or commercial use of the cells may require a separate license and additional fees; contact [sales@bpsbioscience.com](mailto:sales@bpsbioscience.com) for details. Publications using this cell line should reference BPS Bioscience, Inc., San Diego.

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