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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

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

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-α/β 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 ~5 x 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®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 µg/ml of Puromycin (Takara, #631306).

Cells should be grown at 37°C with 5% CO_2 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_2 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 10^6 cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.5 x 10^6 cells/ml. Do not allow the cell density to exceed 2.0 x 10^6 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_2 for 24 hours.

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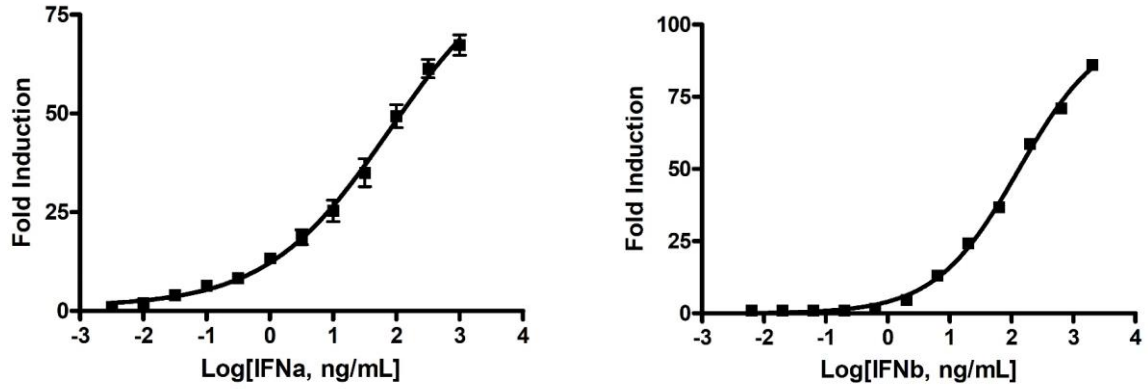
6. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

Figure 1. IFNα/β dose response in IRF reporter (Luc)-THP-1 cells. Cells were treated in assay medium with IFNα or IFNβ 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_2 for 24 hours.

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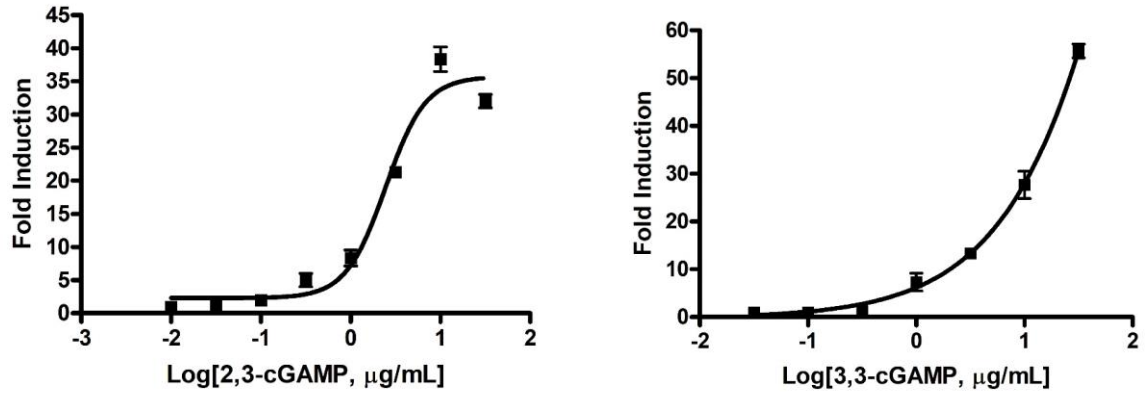
6. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer. Subtract background luminescence value from all readings.

Figure 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

Product	Cat. #	Size
NF-κB 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

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