IRF Reporter (Luc) – THP-1 Cell Line (cGAS-Sting Signaling Pathway)

#79858

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 the 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 the reaction of GTP and ATP to form cGAMP. cGAMP binds to STING (Stimulator of Interferon Genes) which triggers phosphorylation of IRF3 by serine/threonine kinase TBK1 (TANK-binding kinase 1). 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 compound activity.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 x 10^6 cells in 1 ml of cell freezing medium (BPS Bioscience, #79796)

Parental Cell Line

THP-1, human monocyte, suspension

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 the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents 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 below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 8	BPS Bioscience #79652
Growth Medium 8A	BPS Bioscience #79653

Materials Required for Cellular Assay

Name	Ordering Information
IFNα	PBL Assay Science # 11100-1
IFNβ	R&D systems #8499-IF-010
STING agonist: 2'3'-cGAMP	Invivogen #tlrl-nacga23
STING agonist: 3'3'-cGAMP	Invivogen #tlrl-nacga
SR-717	Selleckchem #S0853
GSK8612	Selleckchem #S8872

Assay Medium 2B	BPS Bioscience #79619
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	

Storage Conditions

Cells are shipped in 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. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 8 (BPS Bioscience, #79652):
RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin.

Growth Medium 8A (BPS Bioscience, #79653):
RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin plus 1 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Assay Medium 2B (BPS Bioscience, #79619):
RPMI 1640 medium supplemented with 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 8 **(no Puromycin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.
2. Immediately spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium **8 (no Puromycin)**.
3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium **8 (no Puromycin)]**, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium **8A (contains Puromycin)**.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium **8A (contains Puromycin)**. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 x g for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 cells/ml.
2. Dispense 1 ml of cell aliquots into cryogenic vials. Place the vials in an insulated container for slow cooling and store at -80°C overnight.
3. Transfer the vials to liquid nitrogen the next day for storage.

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

Functional validation

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.

Assay Medium: Assay Medium 2B (BPS Bioscience #79619)

A. Agonist dose response

1. Seed cells at a density of ~40,000 cells per well into a white clear bottom 96-well microplate in 75 µl of assay medium. Leave a few wells empty for determination of background luminescence.
2. Prepare threefold serial dilutions of agonist at 4-fold the final desired concentration in assay medium. Add 25 µl of diluted agonist to treatment wells. The final volume is now 100 µl.
3. Add 25 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.
6. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer. If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.
7. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

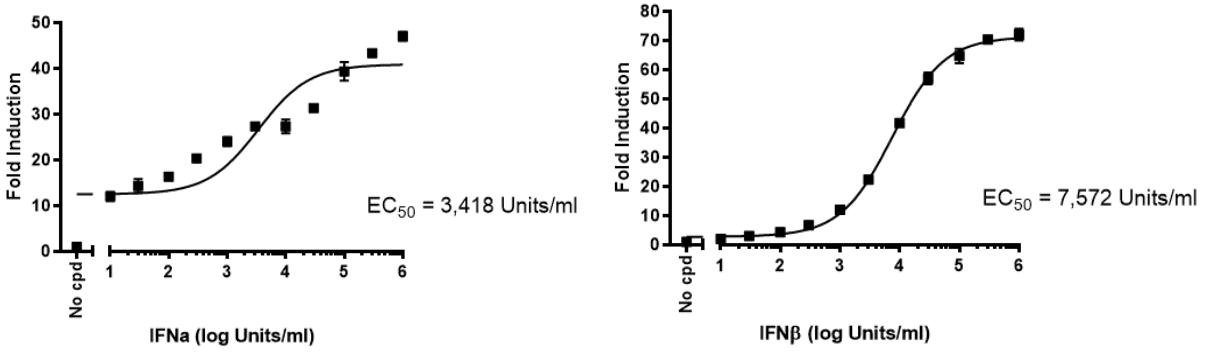

Figure 1. IFNα/β dose response in IRF reporter (Luc)-THP-1 cells. Cells were treated in assay medium with IFNα or IFNβ for 24 hours. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without treatment.

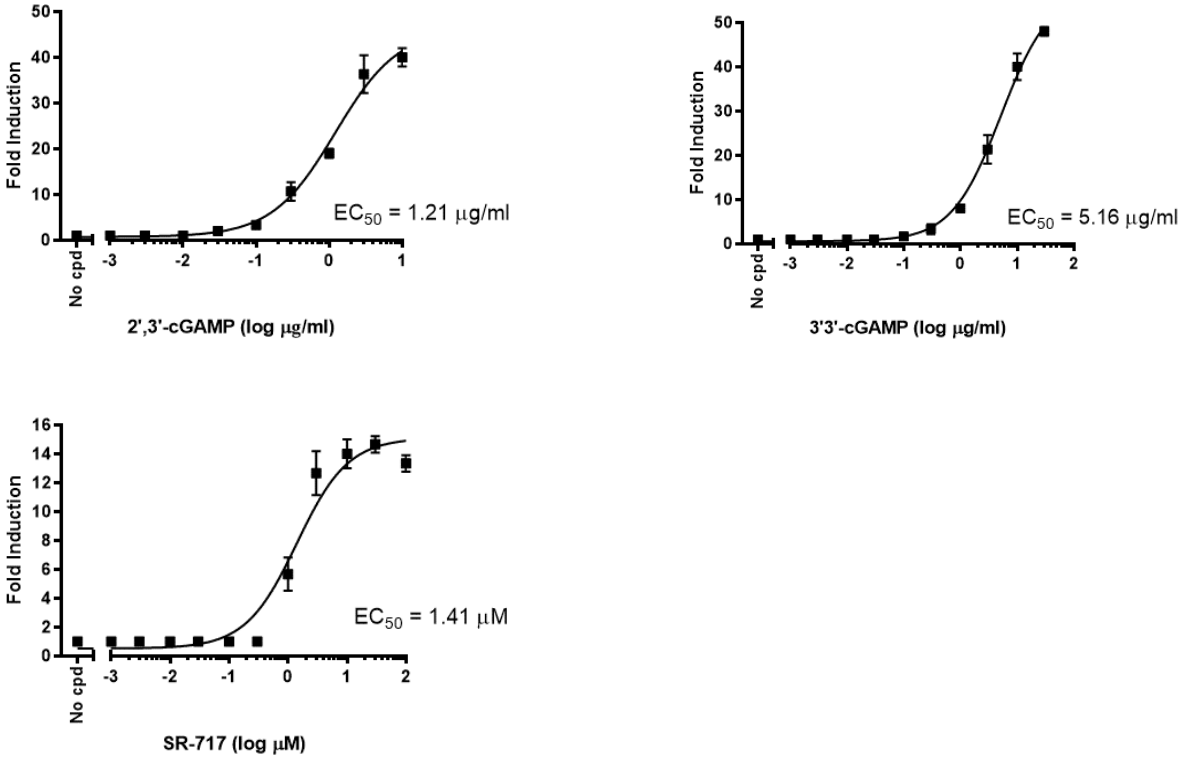

Figure 2. STING agonist dose response in IRF reporter (Luc)-THP-1 cells. Cells were treated with 2'3'-cGAMP, 3'3'-cGAMP or SR-717 in assay medium for 24 hours. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without treatment.

B. Antagonist dose response

1. Seed cells at a density of ~40,000 cells per well into a white clear bottom 96-well microplate in 50 µl of assay medium. Leave a few wells empty for determination of background luminescence.

2. Prepare threefold serial dilutions of antagonist at 4-fold the final desired concentration in assay medium. Add 25 µl of diluted antagonist to treatment wells. Add 25 µl of assay medium to control wells with no antagonist.
3. Incubate at 37°C with 5% CO_2 for 1 hour.
4. Prepare agonist at 4-fold the final desired concentration in assay medium. Add 25 µl of diluted agonist to treated wells. Add 25 µl of assay medium to control wells not treated with agonist to determine the range of the assay. The final volume of the assay is now 100 µl.
   Note 1: the effect of an antagonist can only be observed against stimulation with an agonist.
   Note 2: to determine the range of the assay, several controls are needed: no agonist and no antagonist (0%), agonist without antagonist (100%)
5. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
6. Incubate at 37°C with 5% CO_2 for 24 hours.
7. Prepare ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer. If using other luciferase reagents from other vendors, follow the manufacturer's assay protocol.
8. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

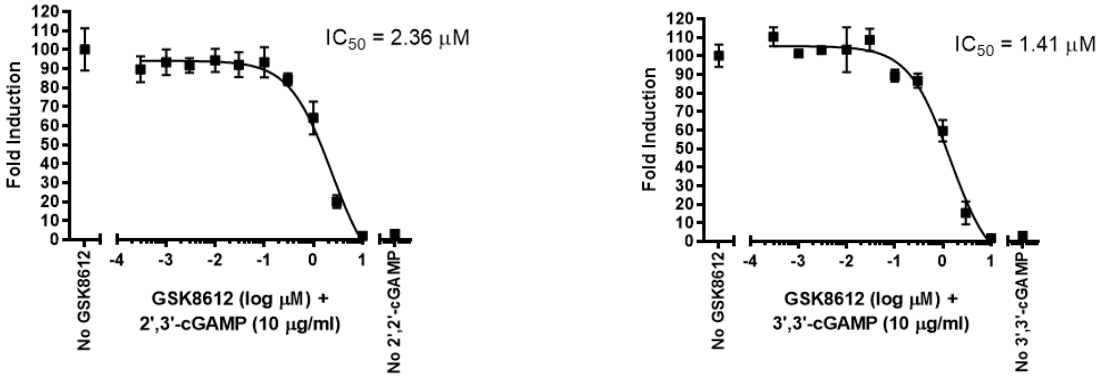

Figure 3. Antagonist dose response in IRF reporter (Luc)-THP-1 cells. Cells were treated with GSK8612 in assay medium for 1 hour followed by treatment with the agonists 2'3'-cGAMP or 3'3'-cGAMP in assay medium for 24 hours. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing values against the mean value for control cells without treatment.

License Disclosure

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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*
NF- κB Reporter (Luc) – THP-1 Cell Line	79645	2 vials
ISRE Reporter-HEK293 Recombinant Cell Line (JAK pathway)	60510	2 vials
ISRE Reporter Kit (JAK/STAT Signaling Pathway)	60613	500 reactions
ISRE Luciferase Reporter Lentivirus (JAK/STAT Signaling Pathway)	79824	500 µl x 2
ONE-Step™ Luciferase Assay System	60690	Multiple Sizes
Thaw Medium 8	79652	100/500 ml
Growth Medium 8A	79653	500 ml
Assay Medium 2B	79619	100/500 ml