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- Diagnostik & molekulare Diagnostik
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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

IRF1 Luciferase Reporter HEK293 Cell Line is a HEK293 cell line that contains a firefly luciferase reporter under the control of three copies of the interferon gamma-activated sites (GAS) of the IRF1 (interferon regulatory factor 1) promoter stably integrated into HEK293 cell line. It is designed to monitor the activity of interferon gamma-induced signal transduction pathways in an HEK293 cell line.

This cell line has been shown to be responsive to IFNγ and IFNα.

Background

IRF1 (interferon regulatory factor 1), also known as MAR, is a transcription factor ubiquitously expressed at low levels, which is responsive to IFN (interferon). It regulates pro-inflammatory responses, cell development and differentiation. Depending on the context, it can act as a tumor suppressor or oncogene, and has been linked also to auto-immune, inflammatory and metabolic disorders. It has been shown that IL-12 induces the binding of STAT4 (Signal transducer and activator of transcription 4) to the GAS (gamma-activated sites) of the IRF1 promoter in Th1 cells resulting in up-regulation of IRF1. On the other hand, IRF1 regulates the expression of PD-L1 (programmed death ligand 1), an immune checkpoint, and it has been linked to the development of resistance to immunotherapy and chemotherapy. IRF1 is a promising therapeutic target in several disorders, and further studies will open new treatment avenues.

Application

- Monitor IFNγ and INFα-induced activity.
- Screen molecules that block the IFN signaling pathway.
- Investigate IL-12 related signaling pathway after introducing STAT4 and IL-12 receptor complex in this cell line.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains ≥1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent.

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 1	BPS Bioscience #60187
Growth Medium 1B	BPS Bioscience #79531

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 1	BPS Bioscience #60187
Recombinant Human IFN-gamma Protein	R&D System #285-IF-100
Recombinant Human IFN-alpha Protein	R&D System #10984-IF-010
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, the use of validated and optimized media from BPS Bioscience is *highly recommended*. 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 to maintain selective pressure on the cell population expressing the gene of interest.

Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 1 (BPS Bioscience #60187):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate and 1% Penicillin/Streptomycin.

Growth Medium 1B (BPS Bioscience #79531):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, and 400 µg/ml of G418.

Media Required for Functional Cellular Assay

Assay Medium: Thaw Medium 1 (BPS Bioscience #60187)
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate and 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 1 to the conical tube containing the cells. Thaw Medium 1 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml, or 12 ml, of pre-warmed Thaw Medium 1.

5. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they are fully confluent. At first passage and subsequent passages, use Growth Medium 1B.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1B and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1B.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 once a week (or 1:5 every 3-4 days).

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1B and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at 1~2 x 10^6 cells/ml.

4. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

5. Transfer the vials to liquid nitrogen the next day for long term storage.

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

Validation Data

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Stimulated Cells" (or "Test Compound"), "Background Control" and "Unstimulated Control" conditions.

A. Dose Response of IRF1 Luciferase Reporter HEK293 Cell Line to IFNγ/IFNα (96-well)

1. Seed cells at a density of 30,000 ~ 40,000 cells per well in 90 µl of Thaw Medium 1 into a white clear-bottom 96-well microplate. Leave a few empty wells as cell-free control wells ("Background Control").
2. Incubate the cells in a 5% CO_2 incubator at 37°C for overnight.
3. Next day, prepare a serial dilution of IFNγ and/or IFNα in Thaw Medium 1 at 10x the final testing concentrations (10 µl/well).
4. Add 10 µl of diluted IFNγ/IFNα to the "Stimulated Cells" wells.
5. Add 10 µl of Thaw Medium 1 to the "Unstimulated Control" wells.
6. Add 100 µl of Thaw Medium 1 to "Background Control" wells (cell-free wells).
7. Incubate at 37°C with 5% CO_2 for ~ 5 hours.
8. Add 100 µl of ONE-Step™ Luciferase reagent per well.
9. Incubate at Room Temperature (RT) for ~10 minutes.
10. Measure luminescence using a luminometer.
11. The "Background Control" luminescence value should be subtracted from all readings.
12. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of luciferase reporter expression is the average background-subtracted

luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

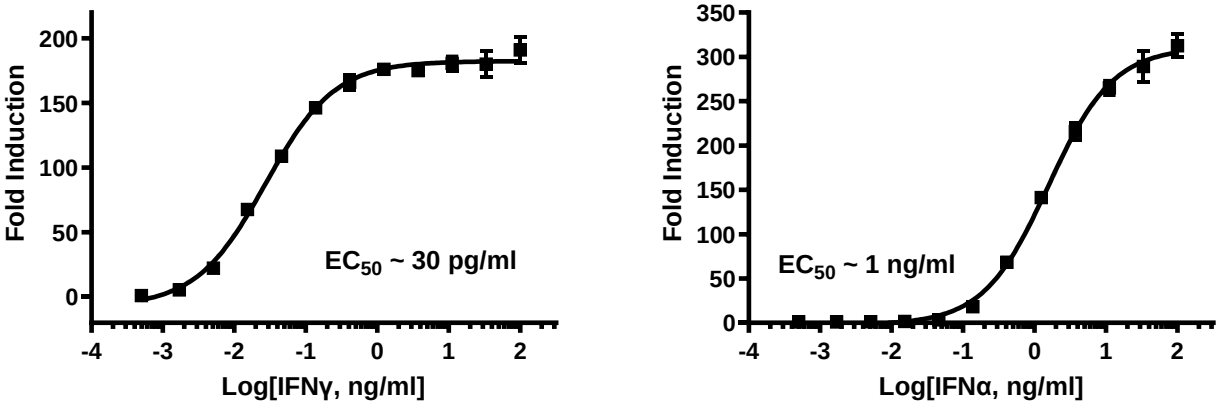

Figure 1. Dose response curve of IRF1 Luciferase Reporter HEK293 Cell Line to IFNγ and IFNα. IRF1 Luciferase Reporter HEK293 cells were treated with increasing concentrations of IFNγ or IFNα. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression in relation to cells without treatment (unstimulated control), and represent four independently performed experiments.

Data shown is representative.

References

Coccia E. M., *et al.,* 1999 *JBC* 274 (10): 6696-6703.
Perevalova A., *et al.*, 2024 *Int J Mol Sci* 25(4):2153.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
IL-15 Responsive Luciferase Reporter Cell Line	78402	2 vials
IL-23 Responsive STAT3 Luciferase Reporter HEK293 Cell Line	82591	2 vials
IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line	78941	2 vials
IL-12 Responsive STAT4 Luciferase Reporter HEK293 Cell Line	82836	2 vials
Human Interleukin-15 Recombinant	90180	2 µg/10 µg
CRE/CREB Luciferase Reporter HEK293 Cell Line (cAMP/PKA Signaling Pathway)	60515	2 vials

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