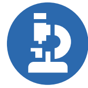
Produktinformation

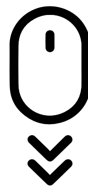
Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
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Description

The STAT3 Reporter Jurkat cell line is designed for monitoring the STAT3 signal transduction pathway. It contains a firefly luciferase gene driven by STAT3 response elements located upstream of the minimal TATA promoter. After activation by cytokines or growth factors, endogenous STAT3 binds to the DNA response elements, inducing transcription of the luciferase reporter gene.

Background

The JAK-STAT signaling pathway is involved in both cancer and autoimmune disease and has been an attractive target for drug discovery. In the context of immune regulation, STAT3 is expressed in diverse cell types. While it was initially described as an acute phase response factor in the context of IL-6 signaling, STAT3 has since been identified as a downstream regulator of many cytokines. STAT3 can also be activated downstream of Type1 Interferons, such as IFN-alpha, where it may function as a negative regulator of Type1 interferon signaling. Studies of the JAK-STAT signaling pathways can prove useful in furthering our understanding of immunological diseases and cancer.

Application

- Screen compound activity on the STAT3 signaling pathway
- Screen inhibitors of STAT3-mediated IL-6 signaling

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

Jurkat (clone E6-1), human T lymphoblast, 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 2	BPS Bioscience #60184
Growth Medium 2K	BPS Bioscience #78078

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Human IFN-alpha A	R&D Systems #11100-1
Pan-JAK inhibitor CP 690,550	Cayman #11598
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	

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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 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 2K (BPS Bioscience #78078):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.25 µg/ml of Puromycin

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 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 2 **(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 2 **(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 2 **(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 2K **(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 0.5 x 10^6 cells/ml of Growth Medium 2K **(contains Puromycin)**. The sub-cultivation ratio should maintain the cells between 0.5 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.

Assay Performance

Functional characterization of STAT3 Reporter Jurkat Cell Line

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:

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

A. Human IFN-alpha dose response

1. Seed the STAT3 Reporter Jurkat cells at a density of 40,000 cells per well into a white, clear-bottom 96-well culture plate in 90 µl of Thaw medium 2. Incubate the cells at 37°C with 5% CO_2 overnight.

2. Add 10 µl/well of a serial dilution of human IFN-alpha (we recommend serial dilutions in 3-fold increments), prepared in Thaw medium 2 at concentrations 10-fold higher than the desired final concentrations, to the wells labeled “hIFN-alpha stimulated”.

   a. Add 10 µl of Thaw medium 2 to unstimulated control wells (for measuring uninduced level of STAT3 reporter activity)

   b. Add 100 µl of Thaw medium 2 to cell-free control wells (for determining background luminescence).

3. Incubate at 37°C with 5% CO_2 for ~5-6 hours

4. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided:

   a. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes.

b. Measure luminescence using a luminometer. If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the average luminescence reading of all wells. The fold induction of STAT3 luciferase reporter expression is the background-subtracted luminescence of hIFN-alpha-stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$fold\ induction = \frac{(luminescence\ stimulated\ cells - background)}{(luminescence\ unstimulated\ cells - background)}$$

Validation Data

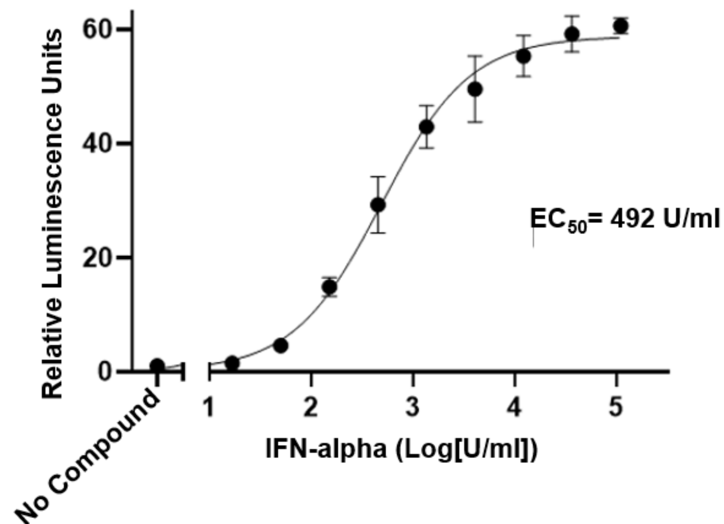

Figure 1: Dose response of STAT3 Reporter Jurkat cells to human IFN-alpha.
Increasing concentrations of human IFN-alpha were added to the cells the day after seeding. Controls consisted of untreated cells and no-cell wells (background). Approximately 5 hours later, the ONEStep™ luciferase assay reagent was added to the cells. 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 hIFN-alpha treatment. The EC_{50} of hIFN-alpha in this cell line was 492 U/ml.

B. Inhibition of hIFN-alpha induced STAT3 Reporter Activity by JAK inhibitor CP 690,550 in STAT3 Reporter Jurkat cells

1. Seed the STAT3 reporter Jurkat cells at a density of 40,000 cells per well into a white clear-bottom 96-well culture plate in 90 µl of Thaw medium 2.

2. Set up each treatment in at least triplicates.

3. Add 10 µl of a serial dilution of CP 690,550 prepared in Thaw medium 2 at concentrations 10-fold higher than the desired final concentrations to wells labeled as "CP 690,550-treated". We recommend preparing a 3-fold increments serial dilution.

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   a. Add 10 µl of Thaw medium 2 to untreated control wells (for measuring uninduced level of STAT3 reporter activity)

4. Incubate the cells at 37°C with 5% CO_2 overnight.

5. Add the following, in triplicate:

   a. Add 10 µl of hIFN-alpha diluted in Thaw medium 2 to wells labeled as "Stimulated" (final hIFN-alpha concentration = 10,000 U/mL)

   b. Add 10 µl of Thaw medium 2 to the unstimulated control wells (for determining the basal activity).

   c. Add 110 µl of Thaw medium 2 to cell-free control wells (for determining background luminescence).

6. Incubate at 37°C with 5% CO_2 for ~5-6 hours

7. Perform luciferase assay using ONE-Step™ Luciferase Assay System according to the protocol provided.

8. Add 110 µl of ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 minutes.

9. Measure luminescence using a luminometer. If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.

10. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the average luminescence reading of all wells. The Relative Luminescence value is the background-subtracted luminescence of CP 690,550-treated cells divided by the background-subtracted luminescence of untreated cells, reported to 100.

$$Relative\ luminescence = \left(\frac{luminescence\ of\ drug\ treated\ cells - background}{luminescence\ of\ untreated\ cells - background}\right) x100$$

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Validation Data

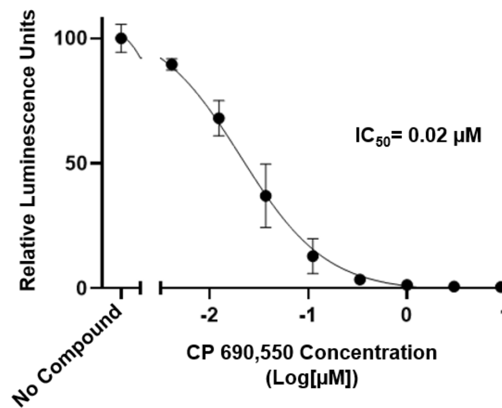

Figure 2: Inhibition of hIFN-alpha induced STAT3 Reporter Activity by JAK inhibitor CP 690,550 in STAT3 Reporter Jurkat cells.
The cells were incubated with increasing concentrations of pan-JAK inhibitor CP 690,550 overnight, then stimulated with human IFN-alpha (10,000 U/ml) for approximately 5 hours. Controls consisted of untreated cells and no-cell wells (background). Luciferase activity was measured using the the ONE-Step™ luciferase assay system. The results are shown as percentage of luminescence of STAT3 reporter activity (in which IFN-stimulated cells in the absence of JAK inhibitor is set at 100%). The IC_{50} of CP 690,550 in this cell line was 0.02 µM.

References

Akira S., *et al* (1994). *Cell* **77(1):**63-71
Hammaren H. M. , *et al.* (2019), *Cytokine* **118:**48-63
Hillmer E, *et al.* (2016). *Cytokine & Growth Factor Review* ***31:*** *1-15*
Tsai M-H, *et al.* (2019). *Front. Immunol.* **10:**1448

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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*
STAT3 Reporter THP-1	78498	500 µl x 2
STAT3 eGFP Reporter Lentivirus	78197	500 µl x 2
STAT3 Luciferase Reporter Lentivirus	79744	500 µl x 2
STAT3 Reporter (Luc) - HEK293 Cell line (Puromycin)	79800-P	2 vials
STAT3 Reporter Kit (STAT3 Signaling Pathway)	79730	500 reactions
STAT3, GST-tag Recombinant	75003	20 µg
Human Interferon-alpha 2a Recombinant	90158-A	20 µg

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