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 that act on the receptor, endogenous STAT3 translocate to the nucleus and binds to the DNA response elements, inducing transcription of the luciferase reporter.

This cell line responds to human interferons IFN-α and IFN-γ but not to interleukins IL-6, IL-1α, IL-2, or TNF-α. Activation of the STAT3 pathway by IFN-α is inhibited by JAK inhibitor CP 690,550.

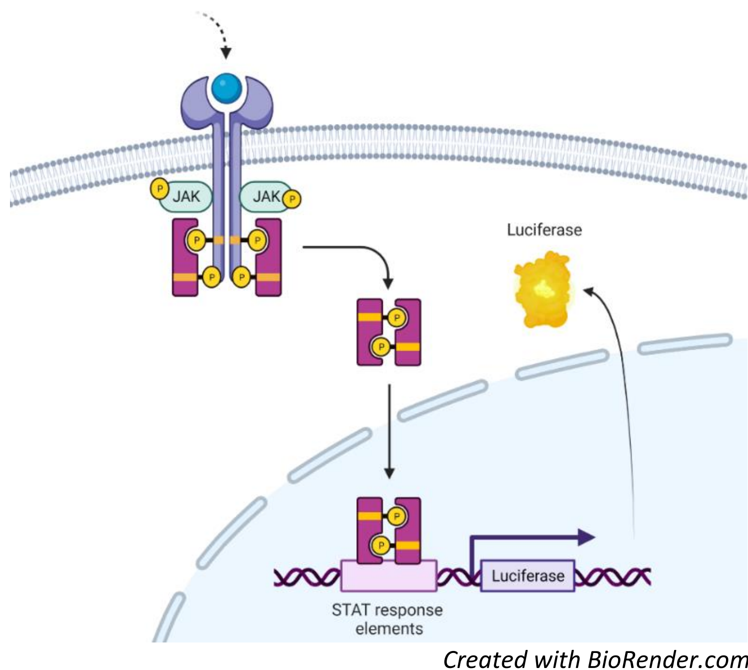

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Figure 1: Mechanism of STAT3 Reporter Jurkat Cell Line response to activation.

Background

The JAK-STAT (Janus kinase signal transducer and activator of transcription) signaling pathway is involved in hematopoiesis, differentiation, metabolism, and immune response, and is responsive to more than 50 cytokines and growth factors. Once a ligand is bound to the receptor, JAKs will recruit STATs. Phosphorylated STATs will dimerize and translocate to the nucleus where they regulate gene transcription. Abnormal JAK-STAT function leads to diseases ranging from rheumatoid arthritis (RA), atopic dermatitis (AD), hematological disorders and other cancer types. This pathway has been an attractive target for drug discovery, with several JAK inhibitors currently approved for clinical use (example, ruxolitinib). 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. Further studies of the JAK-STAT signaling pathways can prove useful in deepening our understanding of immunological diseases and cancer.

Application

Screen compound activity on the STAT3 signaling pathway.

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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

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
Human IFN-γ	R&D Systems #285-IF
IL-6	R&D Systems #206-IL
IL-1α	R&D Systems #200-LA
IL-2	StemCell #78036
TNF-α	R&D Systems #210-TA
Pan-JAK inhibitor CP 690,550	Cayman #11598
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 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

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

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.
   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.

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, and continue growing the cells 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.

Cell Passage

Dilute the cell suspension into new cell culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.5 x 10^6 cells/ml, with Growth Medium 2K. The sub-cultivation ratio used should maintain the cells between 0.5-2 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 Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. 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.

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

A. STAT3 Reporter Jurkat response to various cytokines.

- This experiment measures the effect of an agonist on reporter activation.
- All samples and controls should be performed in triplicate.
- The assay should include “Unstimulated Control”, “Background Luminescence Control” and “Test Condition”.

1. Seed the STAT3 Reporter Jurkat cells at a density of 40,000 cells per well in 90 µl of Thaw Medium 2 into a white, clear-bottom 96-well culture plate. Incubate the cells at 37°C with 5% CO_2 overnight. Keep three wells without cells as “Background Luminescence Control”.

2. Prepare cytokine dilutions (for IFN-α and IFN-γ, we recommend a 3-fold increment serial dilution) in Thaw Medium 2 at concentrations 10-fold higher than the desired final concentrations (10 µl/ well).

3. Add 10 µl of cytokine dilutions to the “Test Condition” wells.

4. Add 10 µl of Thaw Medium 2 to the “Unstimulated Control” wells (for measuring uninduced level of STAT3 reporter activity).

5. Add 100 µl of Thaw Medium 2 to the “Background Luminescence Control” wells.

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

7. Add 100 µl of ONE-Step™ Luciferase reagent per well and rock at Room Temperature (RT) for ~15 minutes.

8. Measure luminescence using a luminometer.

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

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

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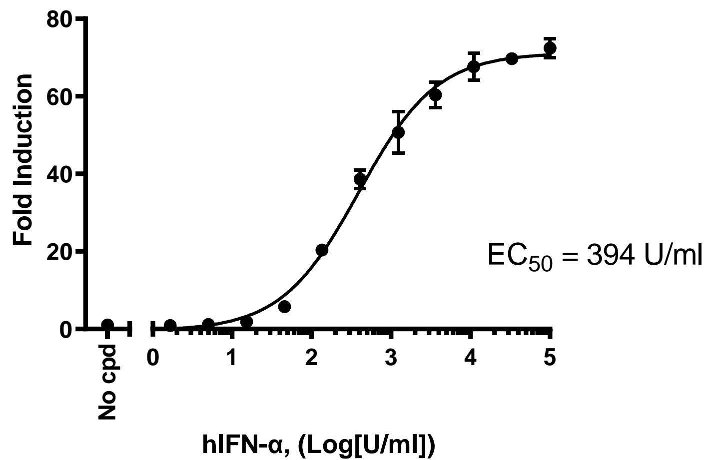

Figure 2: STAT3 Reporter Jurkat cells dose response to human IFN-α.
Cells were treated with increasing doses of human IFN-α for 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of hIFN-α-stimulated cells versus the activity of control cells without hIFN-α treatment (unstimulated control).

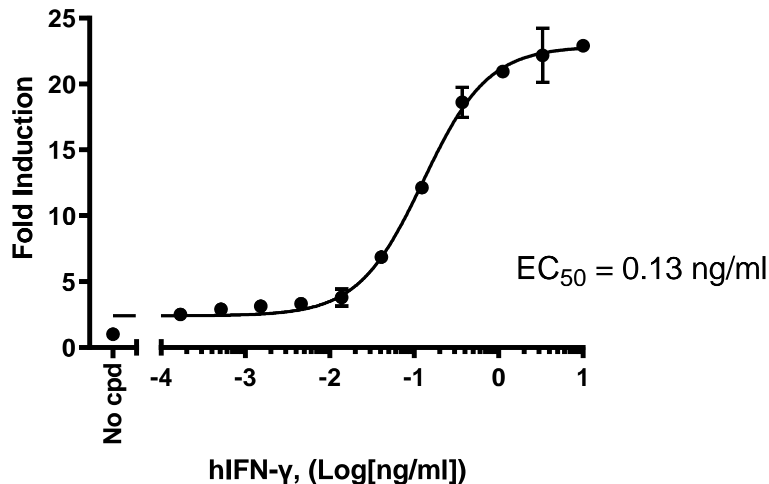

Figure 3: STAT3 Reporter Jurkat cells dose response to human IFN-γ.
Cells were treated with increasing concentrations of human IFN-γ for 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of hIFN-γ-stimulated cells against the activity of control cells without hIFN-γ treatment (unstimulated control).

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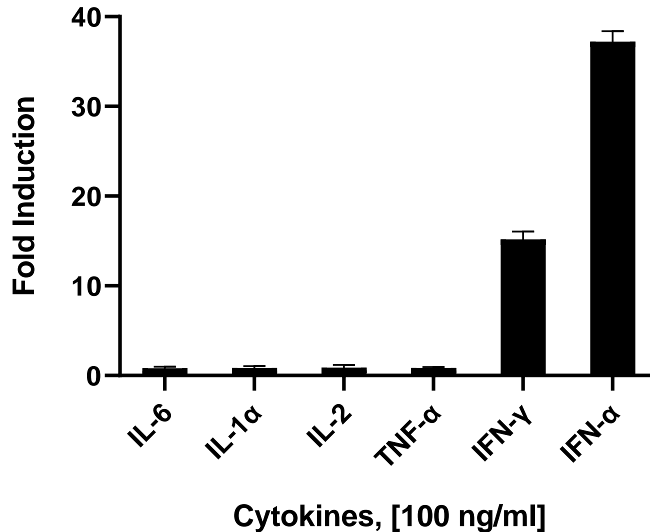

Figure 4: STAT3 Reporter Jurkat cells response to various cytokines.
Cells were treated with 100 ng/ml of IL-6, IL-1α, IL-2, TNF-α, IFN-γ, and IFN-α for 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of treated cells against the activity of the control cells without treatment (unstimulated control).

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

- This experiment measures the effect of an inhibitor against stimulation by hIFN-α.
- All samples and controls should be performed in triplicate.
- The assay should include "Agonist, No Inhibitor Control", "No Agonist, No Inhibitor Control", "Background Luminescence Control" and "Test Condition".

1. Seed the STAT3 reporter Jurkat cells at a density of 40,000 cells per well in 90 µl of Thaw Medium 2 into a white clear-bottom 96-well culture plate. Keep three wells without cells as "Background Luminescence Control".

2. Prepare a 3-fold increment serial dilution of a small molecule inhibitor in Thaw Medium 2 at concentrations 10-fold higher than the final desired concentrations (prepare enough for 10 µl per well).

   For a small molecule inhibitor soluble in DMSO, prepare a stock solution in 100% DMSO at a concentration 1,000x higher than the highest desired final concentration, then dilute 100-fold with Thaw Medium 2 to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 1% DMSO.

   Prepare a serial dilution at concentrations 10-fold higher than the desired final concentrations using Thaw Medium 2 containing 1% DMSO.

   For controls use Thaw Medium 2 containing 1% DMSO (Diluent Solution). The concentration of DMSO should not exceed 0.1% in the final reaction.

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3. Add 10 µl of serially diluted inhibitor to the "Test Condition" wells.

4. Add 10 µl of Diluent Solution to the untreated "Agonist, No Inhibitor Control", "No Agonist, No Inhibitor Control" (for measuring basal level of STAT3 reporter activity), and "Background Luminescence Control" wells.

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

6. The next day, prepare hIFN-α in Thaw Medium 2 at 110,000 U/ml ($[\text{hIFN-}\alpha]_{final}$ = 10,000 U/ml).

7. Add 10 µl of hIFN-α to the "Test Condition" and "Agonist, No Inhibitor Control" wells.

8. Add 10 µl of Thaw Medium 2 to the "No Agonist, No Inhibitor Control" (for determining STAT3 basal activity).

9. Add 100 µl of Thaw Medium 2 to "Background Luminescence Control".

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

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

12. Measure luminescence using a luminometer.

13. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the average luminescence reading of all wells. The percent luminescence of STAT3 luciferase reporter expression is the background-subtracted luminescence of CP 690,550-treated cells divided by the background-subtracted luminescence of the "Agonist, No Inhibitor Control" cells, multiplied by 100.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ drug\ treated\ cells - background}{luminescence\ of\ \text{"}Agonist, No\ Inhibitor\ Control\text{"}\ cells - background}\right) x100$$

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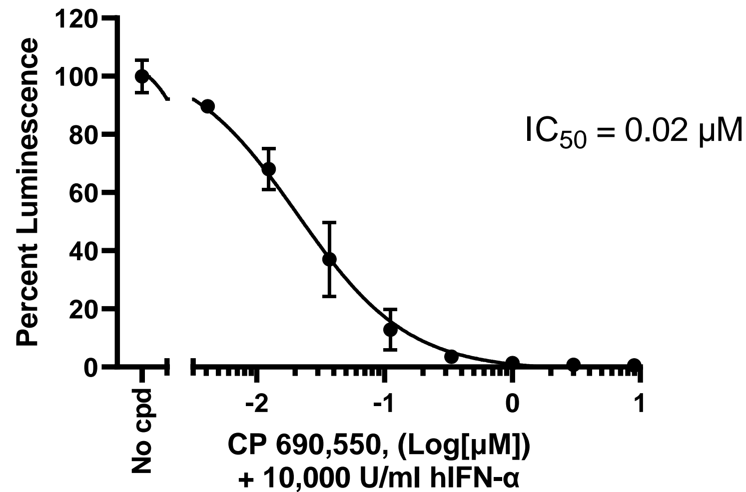

Figure 5: Inhibition of hIFN-α induced STAT3 Reporter Activity by JAK inhibitor CP 690,550 in STAT3 Reporter Jurkat cells.

The cells were incubated with increasing concentrations of pan-JAK inhibitor CP 690,550 overnight, then stimulated with human IFN-α (10,000 U/ml) for approximately 5 hours. Controls consisted of untreated cells and no-cell wells (background). Luciferase activity was measured using the ONE-Step™ luciferase assay system. The results are shown as percentage of luminescence of STAT3 reporter activity (in which hIFN-α-stimulated cells in the absence of JAK inhibitor is set at 100%).

References

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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 Kit (STAT3 Signaling Pathway)	79730	500 reactions
STAT3, GST-tag Recombinant	75003	20 µg