Description

The STAT3 Luciferase Reporter THP-1 cell line monitors the STAT3 (Signal Transducer and Activator of Transcription 3) 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, the endogenous transcription factor STAT3 binds to the DNA response elements, inducing transcription of the luciferase reporter gene.

These cells respond to IL-6 (Interleukin 6), to G-CSF (Granulocyte Colony-Stimulating Factor) and to IFN-γ (Interferon γ).

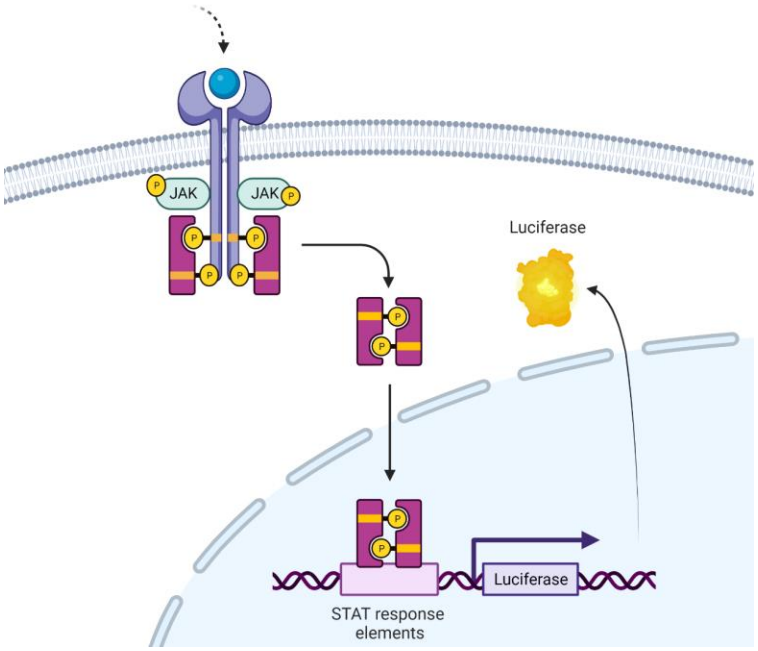

Figure 1: Illustration of STAT3 Luciferase Reporter THP-1 Cell Line reporter expression.
Created with BioRender.com

Background

STAT3 (Signal Transducer and Activator of Transcription 3) is a transcription factor expressed in diverse cell types. STAT3 is phosphorylated by JAK (Janus Kinases) and gets translocated to the nucleus. 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 effector of many cytokines. STAT3-mediated IL-6 signaling in macrophages plays a role in their differentiation and proliferation. The JAK-STAT signaling pathway is involved in both cancer and autoimmune diseases and has been an attractive target for drug discovery in immunological diseases and cancer.

Application

- Monitor STAT3 activity in a monocyte cell line (THP1).
- Screen for compound effect on the STAT3 signaling pathway.
- Screen for inhibitors of STAT3-mediated IL-6 signaling.

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

THP-1, 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
Thaw Medium 8	BPS Bioscience #79652
Recombinant Human IL-6 Protein	R&D Systems #206-IL
IFN-γ	R&D Systems #285-IF
Pan-JAK inhibitor CP 690,550	Cayman #11598
Human IL-6R alpha Antibody	R&D Systems #MAB227
Recombinant Human G-CSF Protein	R&D Systems #214-CS
Anti-GCSFR Antibody (Anumigilimab Biosimilar)	ProteoGenix #PX-TA1776
White, clear-bottom cell culture plate, 96-well	
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 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

Thaw Medium 8 (BPS Bioscience #79652):

RPMI 1640 medium supplemented with 10% heat inactivated FBS, 1% Penicillin/Streptomycin

Cell Culture Protocol

Note: THP-1 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 8.
   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**.**

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, 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.

Cell Passage

Dilute the cell suspension into new 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 of Growth Medium 8A. 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) at ~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 a 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 8 (BPS Bioscience #79652):
RPMI 1640 medium supplemented with 10% heat inactivated FBS, 1% Penicillin/Streptomycin

A. STAT3 Reporter 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 STAT3 Luciferase Reporter THP-1 cells at a density of 60,000 cells/well in 90 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell 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 an IL-6 dose response, we recommend a 3-fold increment serial dilution) in Thaw Medium 8 at concentrations 10-fold higher than the desired final concentrations.

3. Add 10 µl of each IL-6 dilution to "Test Condition".

4. Add 10 µl of Thaw Medium 8 to "Unstimulated Control" (for measuring uninduced level of STAT3 reporter activity).

5. Add 100 µl of Thaw Medium 8 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 for ~15 minutes.

8. Measure luminescence using a luminometer.

9. Data Analysis: Subtract the average background luminescence from the luminescence reading of all conditions. The fold induction of STAT3 luciferase reporter expression is the background-subtracted luminescence of "Test Condition" divided by the background-subtracted luminescence of the "Unstimulated Control".

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

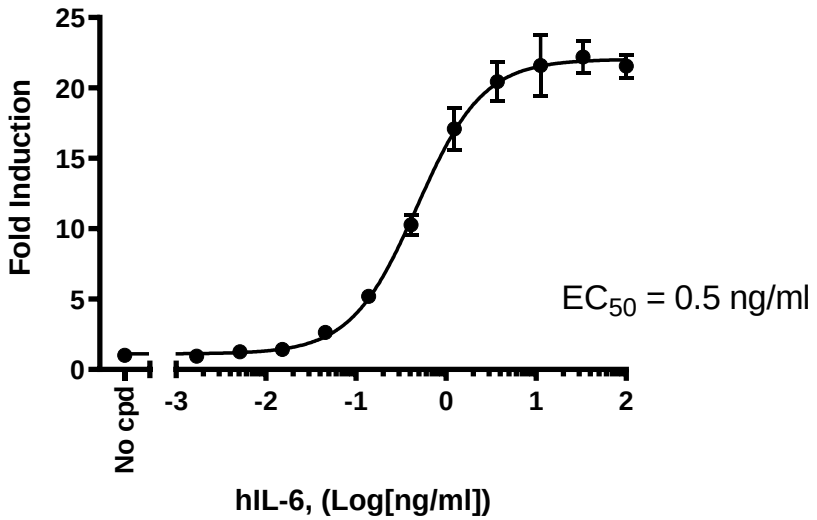

Figure 2: Dose response to human IL-6 in STAT3 Luciferase Reporter THP-1 cells.
Cells were treated with increasing concentrations of human IL-6 (R&D Systems #206-IL) for 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of IL-6-stimulated cells against the activity of the cells without hIL-6 treatment.

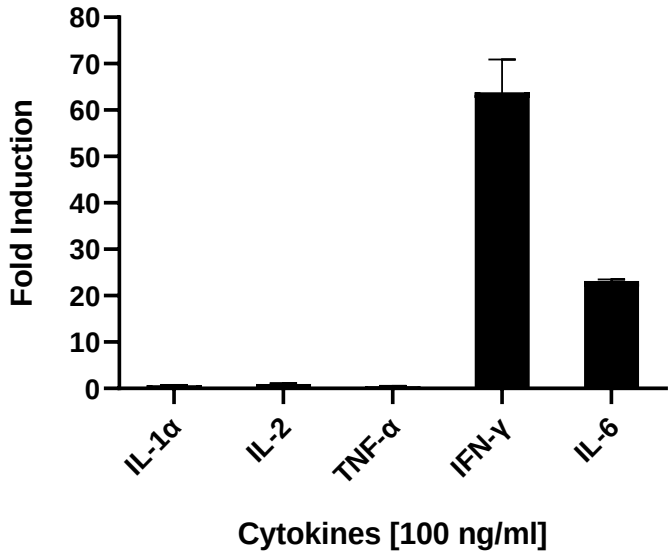

Figure 3: Cytokine response in STAT3 Luciferase Reporter THP-1 cells.
Cells were treated with 100 ng/ml of various cytokines for 5 hours prior to performing the ONE-Step™ luciferase assay. IL-1α (R&D Systems #200-LA), IL-2 (StemCell #78036), TNF-α (R&D Systems #210-TA), IFN-γ (R&D Systems #285-IF), and IL-6 (R&D Systems #206-IL) were tested. 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 cells without treatment.

B. Response to agonist human G-CSF

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

1. Seed STAT3 Luciferase Reporter THP-1 cells at a density of 60,000 cells/well in 90 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Incubate the cells at 37°C with 5% CO_2 overnight. Keep three wells without cells as "Background Luminescence Control".

2. Prepare a 3-fold increment serial dilution of hG-CSF in Thaw Medium 8 at concentrations 10-fold higher than the desired final concentrations.

3. Add 10 µl of each dilution to the "Test Condition".

4. Add 10 µl of Thaw Medium 8 to the "Unstimulated Control" (for measuring uninduced level of STAT3 reporter activity.

5. Add 100 µl of Thaw Medium 8 to the "Background Luminescence Control".

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 for ~15 minutes.

8. Measure luminescence using a luminometer.

9. Data Analysis: Subtract the average background luminescence from the luminescence reading of all conditions. The fold induction of STAT3 luciferase reporter expression is the background-subtracted luminescence of "Test Condition" divided by the background-subtracted luminescence of "Unstimulated Control".

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

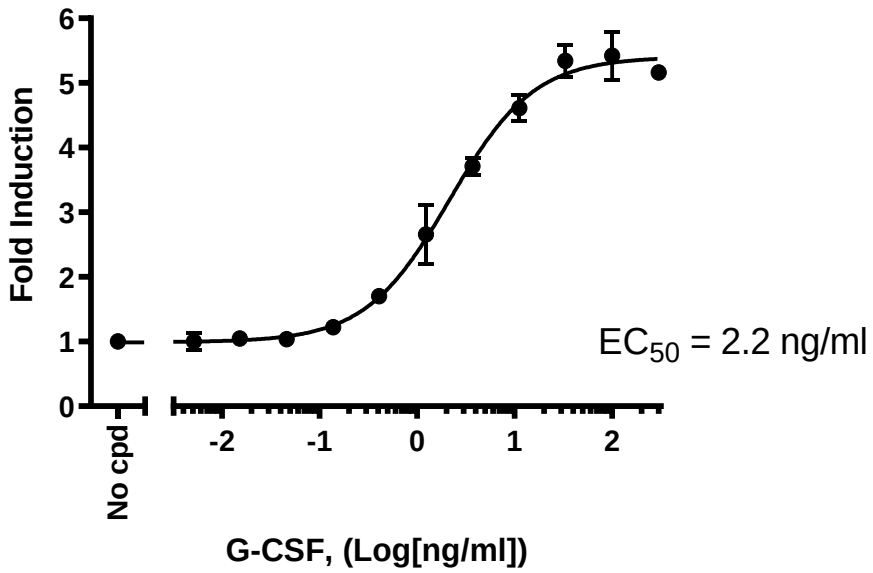

Figure 4: Dose-dependent response to human G-CSF in STAT3 Luciferase Reporter THP-1 cells. Cells were treated with increasing concentrations of human G-CSF (R&D Systems #214-CS) for 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of G-CSF-stimulated cells against the activity of cells without G-CSF treatment.

C. Inhibition of IL-6-induced STAT3 Reporter Activity by a JAK inhibitor (example: CP 690,550) or an IL-6 Receptor Antibody (Anti-IL6R) in STAT3 Luciferase Reporter THP-1 cells

- This experiment measures the effect of a neutralizing antibody or an inhibitor against stimulation by IL-6.
- 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 Luciferase Reporter THP-1 cells at a density of 60,000 cells/well in 75 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Keep three well without cells as "Background Luminescence Control".

2. Prepare a 3-fold increment serial dilution of a small molecule inhibitor or Anti-IL6R antibody at concentrations 4-fold higher than the desired final concentrations (prepare enough for 25 µl/well).

    a. For a small molecule inhibitor soluble in DMSO, prepare a stock solution in 100% DMSO at a concentration 200x higher than the highest desired final concentration, then dilute 50-fold with Thaw Medium 8 to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 2% DMSO.

    Prepare a serial dilution at concentrations 4-fold higher than the desired final concentrations using Thaw Medium 8 containing 2% DMSO.

    For controls use Thaw Medium 8 + 2% DMSO (Diluent Solution). The concentration of DMSO should not exceed 0.5% in the final reaction.

    b. For Anti-IL6R antibody prepare a serial dilution in Thaw Medium 8 at concentrations 4-fold higher than the desired final concentrations. For controls use Thaw Medium 8 (Diluent Solution).

3. Add 25 µl of the serially diluted inhibitors to "Test Condition".

4. Add 25 ml 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 hIL-6 in Thaw Medium 8 at 110 ng/ml ([final on cells] = 10 ng/ml).

7. Add 10 µl of hIL-6 to the "Test Condition" and "Agonist, No Inhibitor Control".

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

9. Add 85 µl of Thaw Medium 8 to "Background Luminescence Control".

	Background Luminescence Control	**Test Inhibitor**	**No Inhibitor Control**	**No Agonist, No Inhibitor Control**
Cell suspension (60,000 cells/ 75 µl)	-	75 µl	75 µl	75 µl
Test Inhibitor	-	25 µl	-	
Diluent Solution	25 µl	–	25 µl	25 µl
Diluted hIL-6		10 µl	10 µl	
Thaw Medium 8	85 µl	-	-	10 µl
Total	**110 µl**	**110 µl**	**110 µl**	**110 µl**

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 Room Temperature for ~15 minutes.

12. Measure luminescence using a luminometer.

13. Data Analysis: Subtract the average background luminescence from the average luminescence reading of all conditions. The percent luminescence of STAT3 luciferase reporter expression is the background-subtracted luminescence of inhibitor-treated cells divided by the background-subtracted luminescence of untreated control cells, multiplied by 100. IL-6-stimulated cells in the absence of JAK inhibitor is set at 100%.

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

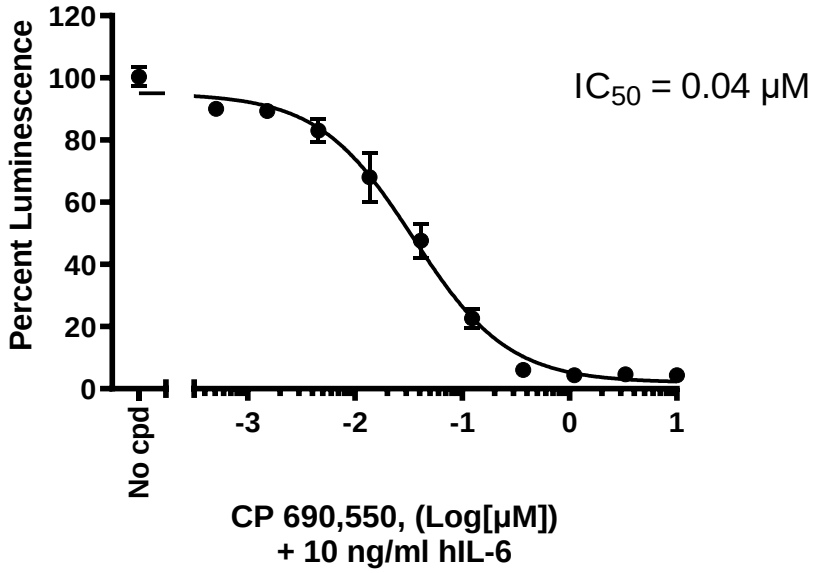

Figure 5: Inhibition of hIL-6-induced STAT3 Reporter Activity by JAK inhibitor CP 690,550 in STAT3 Luciferase Reporter THP-1 cells.
The cells were incubated with increasing concentrations of pan-JAK inhibitor CP 690,550 overnight, then stimulated with human IL-6 (10 ng/ml) for approximately 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as percent luminescence of STAT3 reporter activity (in which IL-6-stimulated cells in the absence of JAK inhibitor is set at 100%).

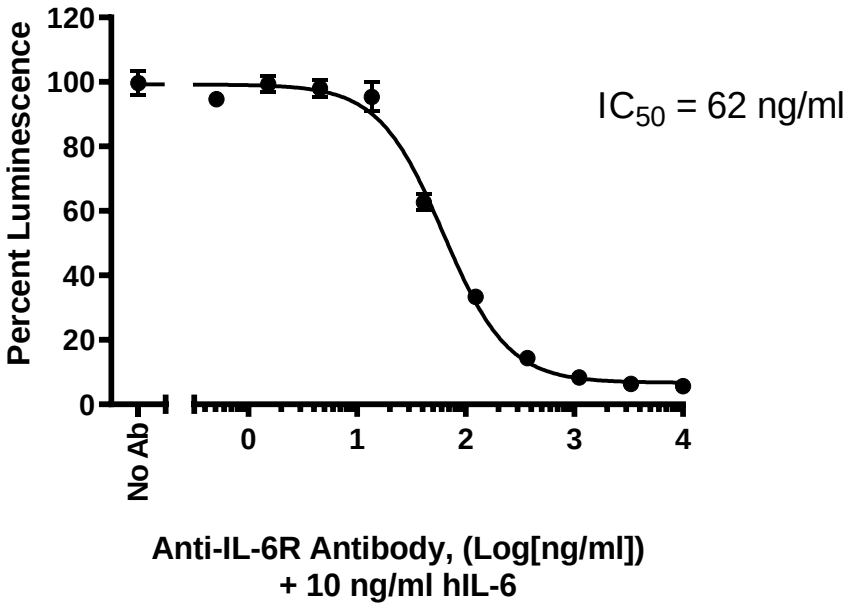

Figure 6: Inhibition of hIL-6-induced STAT3 Reporter Activity by anti-IL-6 Receptor Antibody in STAT3 Luciferase Reporter THP-1 cells.
The cells were incubated with increasing concentrations of anti-IL6R antibody overnight, then stimulated with human IL-6 (10 ng/ml) for approximately 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as percent luminescence of STAT3 reporter activity (in which IL-6-stimulated cells in the absence of anti-IL6R antibody is set at 100%).

D. Inhibition of G-CSF-induced STAT3 Reporter Activity by anti-G-CSF Receptor Antibody (Anti-G-CSFR) in STAT3 Luciferase Reporter THP-1 cells

- This experiment measures the effect of a neutralizing antibody against stimulation by G-CSF.
- All samples and controls should be performed at minimum in triplicate.
- The assay should include "No Inhibitor Control", "No Agonist, No Inhibitor Control", "Background Luminescence Control" and "Test Condition".

1. Seed the STAT3 Luciferase Reporter THP-1 cells at a density of 60,000 cells/well in 75 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Keep three wells without cells as "Background Luminescence Control".

2. Prepare a 3-fold increment serial dilution of Anti-G-CSFR antibody in Thaw Medium 8 at concentrations 4-fold higher than the desired final concentrations (prepare enough for 25 µl/well).

3. Add 25 µl of each dilution to the "Test Condition".

4. Add 25 µl of Thaw Medium 8 to the "No Inhibitor Control", "No Agonist, No Inhibitor Control" (for measuring basal level of STAT3 reporter activity) and "Background Luminescence Control".

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

6. The next day, prepare hG-CSF in Thaw Medium 8 at 110 ng/ml ([final on cells] = 10 ng/ml).

7. Add 10 µl of diluted hG-CSF to the "Test Condition" and "No Inhibitor Control".

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

9. Add 85 µl of Thaw Medium 8 to the "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 Room Temperature for ~15 minutes.

12. Measure luminescence using a luminometer.

13. Data Analysis: Subtract the average background luminescence from the average luminescence reading of all conditions. The percent luminescence value is the background-subtracted luminescence of inhibitor-treated cells divided by the background-subtracted luminescence of "No inhibitor" cells, multiplied by 100. G-CSF-stimulated cells in the absence of anti-G-CSFR antibody is set at 100%.

$$Relative\ luminescence = \left(\frac{luminescence\ of\ inhibitor\ treated\ cells - background}{luminescence\ of\ no\ inhibitor\ cells - background}\right) x100$$

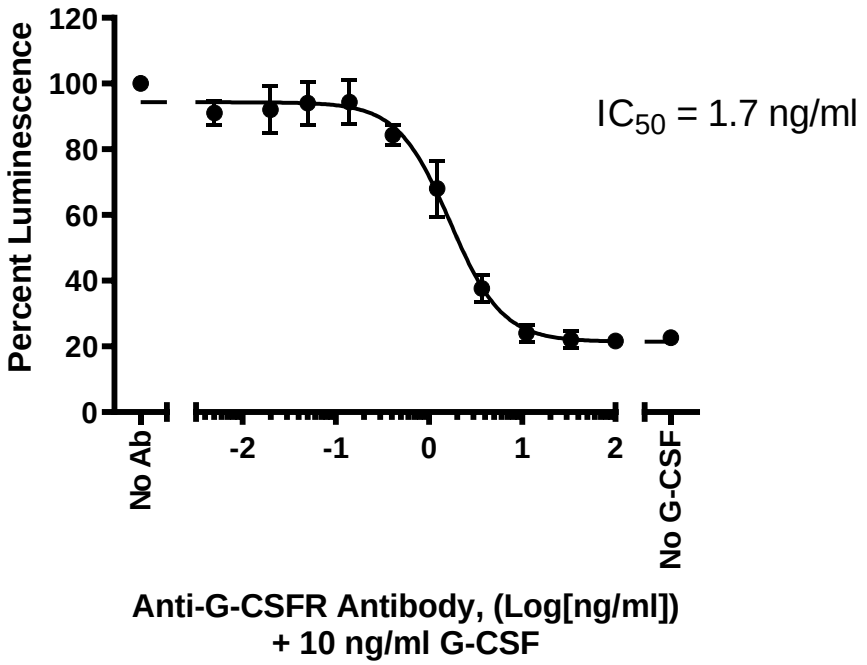

Figure 7: Inhibition of G-CSF-induced STAT3 Reporter Activity by anti-G-CSF Receptor Antibody in STAT3 Luciferase Reporter THP-1 cells.
The cells were incubated with increasing concentrations of anti-G-CSFR antibody overnight, then stimulated with human G-CSF (10 ng/ml) for approximately 5 hours prior to performing the ONE-Step™ luciferase assay. The results are shown as percent luminescence of STAT3 reporter activity (in which G-CSF-stimulated cells in the absence of anti-G-CSFR antibody is set at 100%).

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.
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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 Jurkat Cell Line	78497	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