Description

The STAT5 (Signal transducer and activator of transcription 5) Luciferase Reporter U937 cell line monitors STAT5-mediated signal transduction pathways in the human monocyte cell line U937. It contains a firefly luciferase gene driven by STAT5 response elements located upstream of the minimal TATA promoter. After activation by GM-CSF (Granulocyte-macrophage colony-stimulating factor), the endogenous transcription factor STAT5 binds to the response elements, inducing transcription of the luciferase reporter gene.

These cells respond to GM-CSF. Functional validation experiments showed that GM-CSF-induced luciferase activity was inhibited by anti-GM-CSF neutralizing antibody (Clone #BVD2-21C11).

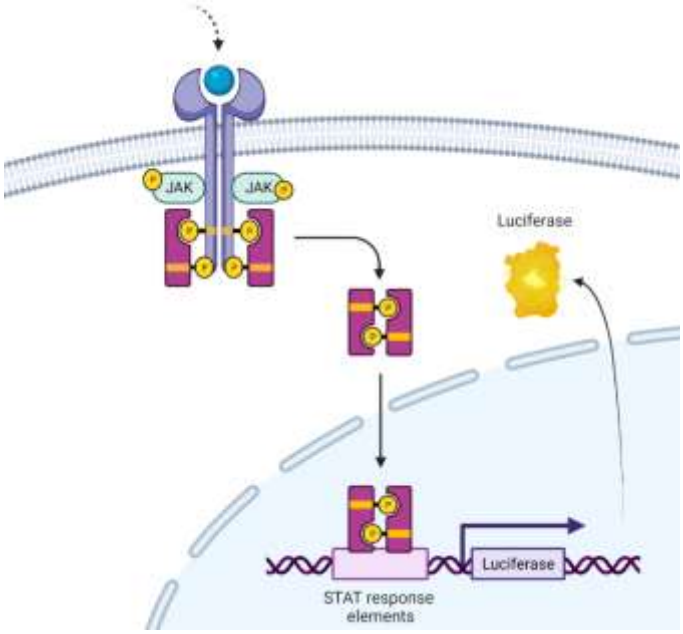

Figure 1: Illustration of STAT5 Luciferase Reporter U937 Cell Line mechanism of action. Created with BioRender.com

Background

GM-CSF is a cytokine that can stimulate the generation of myeloid cells such as granulocytes, neutrophils, and monocytes from its bone marrow precursors. GM-CSF has pro-inflammatory functions and is a therapeutic target in autoimmune disease. GM-CSF signals through JAK2/STAT5 and stimulates the expression of STAT5 target genes. STAT5 includes the highly related proteins STAT5A and STAT5B. These transcription factors mediate cytokine-induced signals from the membrane to the nucleus, regulating the expression of hundreds of specific target genes. Cytokine or growth factor binding to its receptor activates a kinase of the JAK (Janus Kinase) family, which then phosphorylates STAT5 to trigger its dimerization and nuclear translocation. Constitutive phosphorylation of STAT5 is often observed in cancer.

Application

- Monitor STAT5 activity.
- Screen for compounds regulating the STAT5 signaling pathway.
- Study the effect of GM-CSF neutralizing antibodies on STAT5 activity.

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)

Host Cell

U937, human monocyte cell derived from lymphoma, 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.

Materials 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
Recombinant Human GM-CSF	Biolegend #572903
Anti-GM-CSF neutralizing antibody	Biolegend #502319 (clone #BVD2-21C11)
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
White, clear-bottom 96-well tissue culture plate	Corning #3610
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.

Assay Medium: Thaw Medium 8 (BPS Bioscience #79652)
RPMI 1640 medium supplemented with 10% heat-inactivated 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 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 0.1 x 10^6 cells/ml of Growth Medium 8A. The sub-cultivation ratio should maintain the cells between 0.1 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 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.

Validation Data

The following assays are designed for a 96-well format. To perform the assay in a different format, the cell number and reagent volume should be scaled appropriately.

A. Activation of STAT5 Luciferase Reporter U937 cells by human GM-CSF

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

1. Seed cells at a density of ~40,000 cells/well in 90 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Keep three wells without cells as "Background Luminescence".

2. Prepare a 3-fold increment serial dilution of GM-CSF in Thaw Medium 8 at concentrations 10-fold higher than the desired final concentrations (10 µl/well).

    a. Add 10 µl of diluted GM-CSF to "Stimulated".

    b. Add 10 µl of Thaw Medium 8 to "Unstimulated".

    c. Add 100 µl of Thaw Medium 8 to "Background Luminescence".

3. Incubate the plate overnight at 37°C in a CO_2 incubator.

4. Add 100 µl/well of ONE-Step™ Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

5. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The fold induction of Luciferase reporter expression is the background-subtracted luminescence of stimulated cells divided by the background-subtracted luminescence of unstimulated control wells.

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

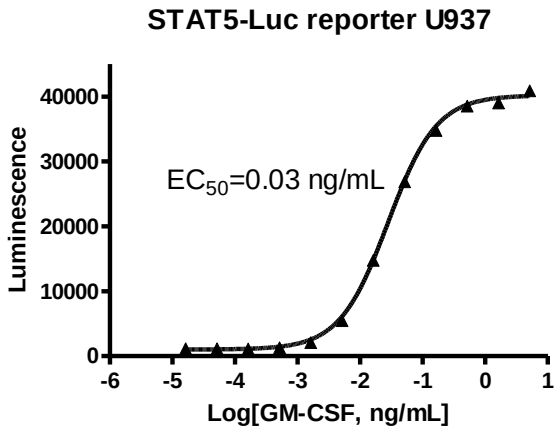

Figure 2. Response of STAT5 Luciferase Reporter U937 Cell Line to human GM-CSF.
Cells were incubated with increasing concentrations of human GM-CSF for approximately 16 hours and luciferase activity was measured using the One-Step™ Luciferase Assay System. Results are shown as Fold Induction (where the unstimulated condition is set to 1).

B. Inhibition of GM-CSF-induced STAT5 activity by an anti-GM-CSF neutralizing antibody

- This experiment measures the effect of an anti-GM-CSF neutralizing antibody against stimulation by GM-CSF.
- All samples and controls should be performed in triplicate.
- The assay should include "Stimulated, No Antibody", "Unstimulated, No Antibody", "Background Luminescence" and "Test Antibody" conditions.

1. Seed cells at a density of ~40,000 cells/well in 80 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Keep three wells without cells as "Background Luminescence".

2. Prepare a serial dilution of anti-GM-CSF antibody (we recommend a 3-fold increment serial dilution) in Thaw Medium 8 at concentrations 10-fold higher than the desired final concentrations (10 µl/well). Add 10 µl of the diluted anti-GM-CSF to "Test Antibody".

3. Prepare a dilution of human GM-CSF in Thaw Medium 8 at a concentration of 3 ng/ml (10-fold the final desired concentration of 0.3 ng/ml).

    a. Add 10 µl of diluted human GM-CSF to ""Test Antibody" and "Stimulated, No Antibody".

    b. Add 10 µl of Thaw Medium 8 to "Unstimulated, No Antibody" (for determining STAT5 basal activity).

    c. Add 100 µl of Thaw Medium 8 to "Background Luminescence" (no cells).

4. Incubate the plate at 37°C with 5% CO_2 for 16 to 24 hours.

5. Add 100 µl/well of ONE-Step™ Luciferase reagent. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

6. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of antibody-treated cells divided by the background-subtracted luminescence of untreated control cells, multiplied by 100.

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

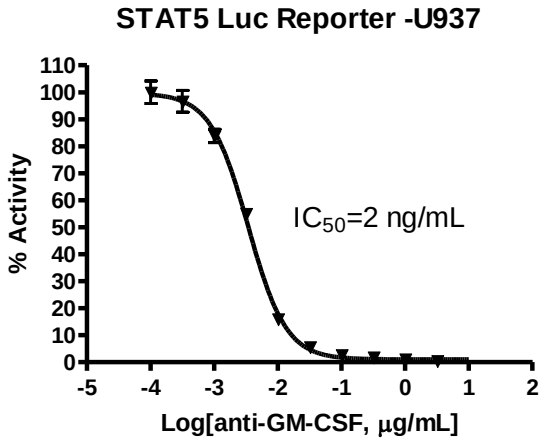

Figure 3. Inhibition of GM-CSF-stimulated STAT5 signaling by anti-GM-CSF antibody (Clone #BVD2-21C11).
Cells were incubated with increasing concentrations of neutralizing antibody before stimulation with 0.3 ng/ml GM-CSF. Luciferase activity was measured using One-Step™ luciferase assay system. Results are shown as percentage of STAT5 reporter activity (compared to cells stimulated by GM-SCF in the absence of antibody, set at 100%).

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Related Products

Products	*Catalog #*	*Size*
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x 2
STAT5 Luciferase Reporter Ba/F3 Cell Line	79772	2 vials
IL-15 Responsive Luciferase Reporter Cell Line	78402	2 vials
STAT5 Peptide	79864	500 µg