Description

The STAT5 Reporter (Luc)-U937 cell line is designed for monitoring STAT5 signal transduction pathway in the U937 cell line. It contains a firefly luciferase gene driven by the STAT5 response element located upstream of the minimal TATA promoter. After activation by GM-CSF, endogenous STAT5 binds to the DNA response elements, inducing transcription of the luciferase reporter gene.

Background

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine stimulating the production of granulocytes and monocytes from bone marrow precursors. GM-CSF had 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.

Application

1. Screen for activators or inhibitors of the STAT5 signaling pathway
2. Screen for the neutralization antibody of GM-CSF

Materials Provided

Components	Format
2 vials of frozen cells	2 x 10^6 cells in 1 ml of 10% DMSO in FBS

Host Cell

U937

Mycoplasma Testing

The cell line has been screened using the MycoAlert™ Mycoplasma Detection kit (Lonza, #LT07-218) to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems 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.

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, clone #BVD2-21C11	Biolegend, #502319
ONE-Step™ Luciferase Assay System	BPS Bioscience, #60690
Luminometer	

Storage Conditions

Cells will arrive upon 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. To formulate a comparable but not BPS-validated medium, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used for maintaining cell lines over many passages. Cells should be grown at 37°C with 5% CO_2 using Growth Medium 8A.

Media Required for Cell Culture

Thaw Medium 8 (BPS Bioscience, #79652):
RPMI 1640 medium (Thermo Fisher, #A1049101) supplemented with 10% heat-inactivated FBS (Thermo Fisher, #10082147), 1% Penicillin/Streptomycin (Hyclone, #SV30010.01).

Growth Medium 8A (BPS Bioscience #79653):
Thaw Medium 8 (BPS Bioscience, #79652) plus 1 µg/ml of Puromycin (Takara, #631306).

Assay Medium: Thaw Medium 8 (BPS Bioscience, #79652)

Cell Culture Protocol

Cell Thawing

1. To thaw the cells, it is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 8 (**no Puromycin**).
2. Spin down the cells, remove supernatant and resuspend cells in 5 ml of pre-warmed Thaw Medium 8 (**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, add an additional ~3 ml of Thaw Medium 8 (**no Puromycin**), and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split.
5. Cells should be split before they are fully confluent. At first passage, switch to Growth Medium 8A (**contains Puromycin**).

Cell Passage

Dilute cell suspension into new culture vessels at no less than 0.1 x 10^6 cells/ml. Do not allow the cell density to exceed 2.0 x 10^6 cells/ml

Cell Freezing

Spin down cells and resuspend cell pellet in 4°C Freezing Medium (BPS Bioscience, #79796) to ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. The next day, transfer to liquid nitrogen for 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.

Figures & Validation Data

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

A. Dose response of STAT5 reporter (Luc) -U937 cells to human GM-CSF

1. Harvest STAT5 reporter (Luc)-U937 cells from culture in the Growth Medium 8A and seed cells at a density of ~40,000 cells per well into a white clear-bottom 96-well microplate in 90 µl of the Assay medium (see above). Leave a couple of wells empty for use as the cell-free control.

2. Prepare threefold serial dilution of GM-CSF in assay medium. Set up each treatment in at least triplicate.
   a. Add 10 µl of diluted GM-CSF to GM-CSF stimulated wells.
   b. Add 10 µl of assay medium to the unstimulated control wells.
   c. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

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

d. Perform luciferase assay using ONE-Step Luciferase Assay buffer, according to the recommended instructions. Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

e. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

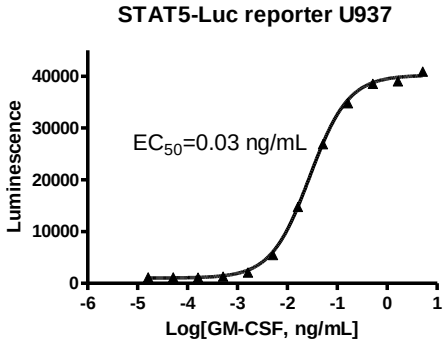

Figure 1. Dose response of STAT5 reporter (Luc) -U937 cells to human GM-CSF

B. Inhibition of GM-CSF induced STAT5 signaling in STAT5 Reporter (Luc) -U937 cells

1. Harvest STAT5 reporter (Luc)-U937 cells from culture in the Growth Medium 8A and seed cells at a density of ~40,000 cells per well into a white clear-bottom 96-well microplate in 80 µl of the Assay Medium. Leave a couple of wells empty as a cell-free control.

2. Prepare serially diluted 10x anti-GM-CSF antibody in the Assay Medium. Add 10 µl of the diluted anti-GM-CSF into each well.

3. Set up each treatment in at least triplicates:
   a. Add 10 µl of diluted human GM-CSF in assay medium to stimulated wells (final GM-CSF concentration = 0.3 ng/ml)
   b. Add 10 µl of assay medium to the unstimulated control cells (for determining the basal activity)
   c. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

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

4. Perform luciferase assay using ONE-Step™ Luciferase Assay kit, according to the recommended instructions. Add 100 µl of the final ONE-Step™ Luciferase reagent per well and rock at room temperature for ~15 to 30 minutes. Measure luminescence using a luminometer.

5. Data Analysis: Subtract average background luminescence (cell-free control wells) from luminescence reading of all wells.

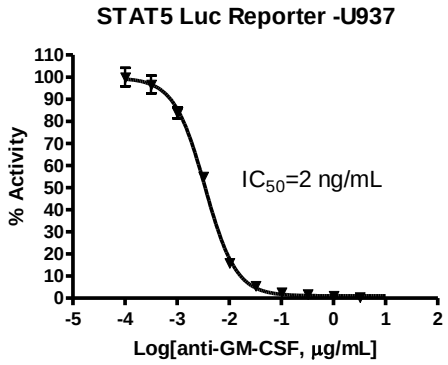

Figure 2. Inhibition of STAT5 signaling by anti-GM-CSF antibody (Clone #BVD2-21C11) in STAT5 reporter (Luc) - U937 cells. Cells were stimulated with 0.3 ng/ml of GM-CSF.

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Related Products

Products	*Catalog #*	*Size*
STAT5 Luciferase Reporter Lentivirus	79745	2 vials
STAT5 Reporter (Luc)-Ba/F3 Cell Line	79772	2 vials