Description

The STAT5 (Signal Transducer and Activator of Transcription 5) Luciferase Reporter murine Ba/F3 cell line monitors STAT5-mediated signal transduction pathways. It contains a firefly luciferase gene driven by STAT5 response elements located upstream of a minimal TATA promoter. After activation by relevant cytokines or growth factors, endogenous STAT5 binds to the response elements, inducing transcription of the luciferase reporter gene.

This cell line responds to mouse interleukin-3 (IL-3).

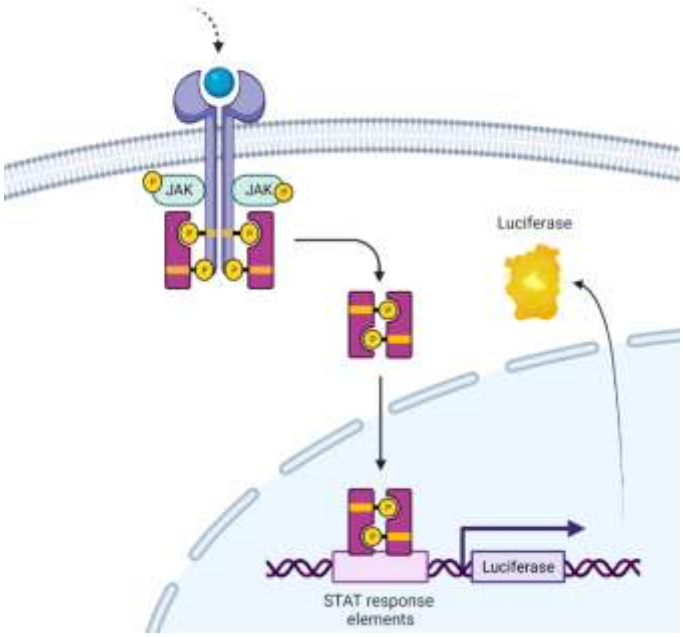

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Figure 1: Illustration of STAT5 Luciferase Reporter Ba/F3 Cell Line.

Background

Signal transducer and activator of transcription 5 (STAT5) includes the highly related proteins STAT5A and STAT5B. These transcription factors relay 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. STAT5 is involved in the development and function of B and T cells. Constitutive phosphorylation of STAT5 is often observed in cancer. The use of inhibitors of the JAK/STAT pathways are potential candidates for the treatment of inflammation related diseases.

Application

- Monitor STAT5 activity.
- Screen and characterize compounds that regulate 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)

Parental Cell Line
Ba/F3, mouse IL-3-dependent pro-B cell line, 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
Mouse IL-3	BPS Bioscience #90189
Growth Medium 8A	BPS Bioscience #79653

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 8	BPS Bioscience #79652
Mouse IL-3	BPS Bioscience #90189
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 these 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) and mouse IL-3:
RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin and 5 ng/ml mouse IL-3.

Growth Medium 8A (BPS Bioscience #79653) and mouse IL-3:
RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin, 1 µg/ml of Puromycin, and 5 ng/ml mouse IL-3.

Note: Mouse IL-3 is essential for Ba/F3 cell maintenance. Thaw Medium 8 and Growth Medium 8A do not contain IL-3.

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

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 **plus IL-3**.

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 **plus IL-3** 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 **plus IL-3**.

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 0.1 x 10^6 cells/ml of Growth Medium 8A **plus IL-3**. 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 storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

A. STAT5 Reporter response to mIL-3

- 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" control.

1. From a cell culture, wash cells once in Thaw Medium 8 (without IL-3). Seed the cells at a density of 20,000 cells/well in 90 µl of Thaw Medium 8 (**without IL-3)** 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 mIL-3 in Thaw Medium 8 at concentrations 10-fold higher than the desired final concentrations (10 µl/well).

3. Add 10 µl of each dilution to "Stimulated".

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

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

6. Incubate at 37°C with 5% CO_2 for 5 to 16 hours.

7. Add 100 µl/well of ONE-Step™ Luciferase reagent.

8. Incubate at room temperature for ~15 minutes.

9. Measure luminescence using a luminometer.

10. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The fold induction of STAT5 luciferase reporter expression is the background-subtracted luminescence of stimulated cells divided by the background-subtracted luminescence of unstimulated control wells.

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

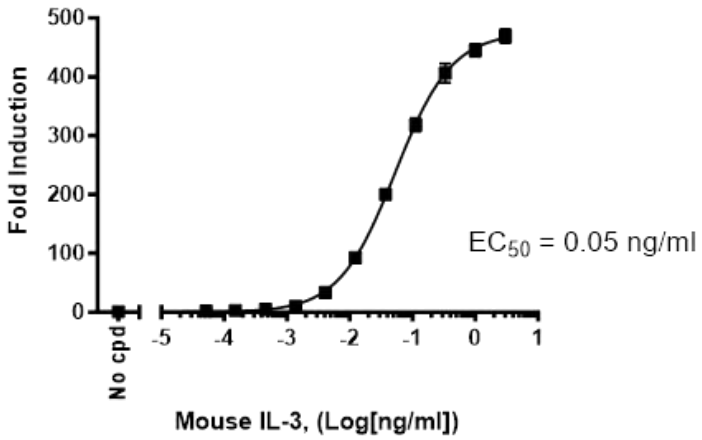

Figure 2: Dose-dependent response of STAT5 Luciferase Reporter Ba/F3 Cell Line to mouse IL-3. Cells were incubated with increasing concentrations of mouse IL-3 for approximately 16 hours and luciferase activity was measured using the One-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of mIL-3-stimulated cells against the activity of cells without mIL-3.

References

Palacios R., *et al.,* 1984 *Nature*, 309 (5964): 126-131.
Jaster R., *et al.,* 1999 *Cell Signal.*, 11(5): 331-335.

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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*
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x 2
STAT5 Peptide	79864	500 µg
STAT5 Luciferase Reporter U937 Cell Line	79941	2 vials
IL-15 Responsive Luciferase Reporter Cell Line	78402	2 vials