Description

The TSLP Responsive Luciferase Reporter Ba/F3 Cell Line is a murine Ba/F3 cell line engineered to express both TSLP-R (thymic stromal lymphopoietin receptor, also known as CRLF2, cytokine receptor like factor 2) (NM_022148.4) and IL-7Ra (Interleukin 7 receptor alpha) (NM_002185.5) separated by a self-cleaving P2A peptide. The construct was delivered by lentiviral transduction of STAT5 Luciferase Reporter Ba/F3 cells (#79772), which express a firefly luciferase reporter driven by STAT5 (signal transducer and activator of transcription 5) response elements located upstream of the minimal TATA promoter. After activation by TSLP, the endogenous transcription factor STAT5 binds to the response elements, inducing transcription of the luciferase reporter.

This cell line has been validated to respond to TSLP. Additional functional validation demonstrates that TSLP-induced luciferase activity can be inhibited by either anti-TSLP or anti-TSLPR neutralizing antibodies. It was also validated with the anti-JAK1/2 inhibitor Ruxolitinib.

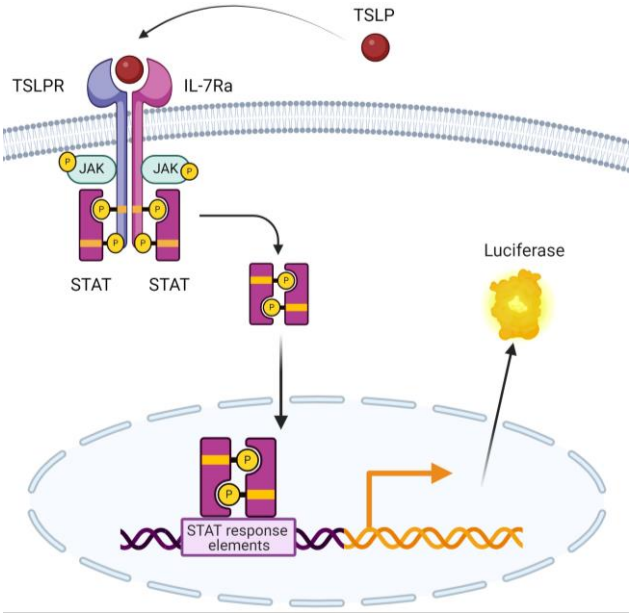

Figure 1: Illustration of the mechanism of TSLP Luciferase Reporter Ba/F3 Cell Line.
TSLP binds to TSLPR, recruits the IL-7Ra co-receptor and activates downstream JAK1/2 (janus kinase 1/2) tyrosine kinases, which phosphorylate the transcription factor STAT5. STAT5 phosphorylation triggers the formation of a homodimer and translocation to the nucleus, where it can activate the transcription of the Firefly luciferase reporter driven by STAT5 response elements present in the promoter.

Background

TSLP (thymic stromal lymphopoietin) is a protein that functions as a type I cytokine, as an alarmin and growth factor in the immune system. It is involved in type 2 immune responses, T_H2 (T helper 2 cells) responses, and the maturation and recruitment of dendritic cells (DCs), T cells, B cells, neutrophils, mast cells, and other lymphoid cells. It can be produced by epithelial and stromal cells in lung, skin, and gastric system, but also by DCs, basophils and mast cells. Its expression can be induced by infections, pro-inflammatory cytokines, proteases, and even mechanical injury. For instance, it can be produced in the lungs in response to infection with influenza or rhinovirus. Its role as alarmin can result in increasing inflammation. TSLP is linked to allergic reactions such as asthma, atopic dermatitis, and food allergies, by inducing the expression of OX40L, CD80 and CD86 and stimulating $CD4^+$ T cells. TSLP signals through a heterodimeric receptor of TSLP-R (CRLF2) and IL7Ra (CD127), and via downstream activation of JAK2/STAT5 signaling. In 2021, the TLSP-neutralizing antibody tezepelumab was

approved for the treatment of severe asthma. Targeting TSLP is an active area of investigation with ongoing clinical trials for the treatment of autoimmune disorders.

Application
Screen and characterize modulators of TSLP 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 Interleukin-3 Recombinant	BPS Bioscience #90189
Growth Medium 8C	BPS Bioscience #82505

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 8	BPS Bioscience #79652
TSLP (R127A, R130A), Avi-His-Tag Recombinant	BPS Bioscience #102201
Anti-TSLP Neutralizing Antibody	BPS Bioscience #102138
Ruxolitinib	BPS Bioscience #82621
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℃ 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

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

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

Complete Growth Medium: Growth Medium 8C (BPS Bioscience #82505) with mouse IL-3 (BPS Bioscience #90189):
RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin, 1 µg/ml of Puromycin, 800 µg/ml hygromycin and 5 ng/ml mouse 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.

   Note: 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 Complete Thaw Medium.

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 Complete Thaw Medium 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 Complete Growth Medium.

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 in Complete Growth Medium. 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

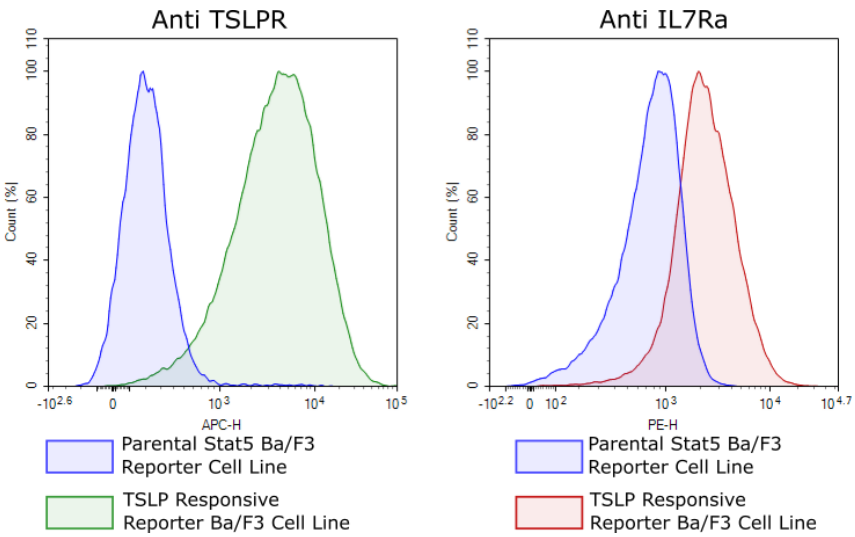

Figure 2: Flow cytometry analysis of TSLPR and IL-7Ra expression in TSLP Responsive Luciferase Reporter Ba/F3 Cell Line.
TSLP Responsive Luciferase Reporter Ba/F3 cells and parental STAT5 Luciferase Reporter Ba/F3 cells (#79772) were stained with either APC anti-human TSLPR (TSLP-R) Antibody (BioLegend #322808) or CD127 Recombinant Rabbit Monoclonal Antibody (001), PE (Thermo Fisher #MA5-46701) and analyzed by flow cytometry. The y axis represents the % of cells, while the x axis represents the fluorophore intensity.

Functional Validation

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

A. Agonist evaluation

- This experiment measures the effect of an agonist on reporter activation.
- The assay should include "Stimulated", "Unstimulated" (negative control, no agonist) and "Background Luminescence" (no cells) conditions.

1. Count cells and determine the cell density.
    a. If the cell density is <1.5 x 10^6 cells/ml, proceed with assay as below.
    b. If the cell density is >1.5 x 10^6 cells/ml, dilute cells in fresh Growth Media 8C.

    **Note: it is critical to start with a healthy cell population for this assay. Beginning with over-confluent cells will substantially reduce the signal window for the assay.*

2. Collect cells from culture and wash cells once with Thaw Medium 8.

3. Seed cells at a density of 50,000 cells/well in 90 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Keep a few empty wells as "Background Luminescence" control.

4. Incubate at 37°C with 5% CO_2 for ~40 hours.

    Note: It is critical to include a ~40-hour starvation in Thaw Media without mIL3 to reduce background signal (as mIL3 also stimulates the STAT5 luciferase reporter).

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

6. Add 10 µl of each dilution to "Stimulated" wells.

7. Add 10 µl of Thaw Medium 8 to "Unstimulated" wells.

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

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

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

11. Incubate at room temperature (RT) for ~15 minutes.

12. Measure luminescence using a luminometer.

13. 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.

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

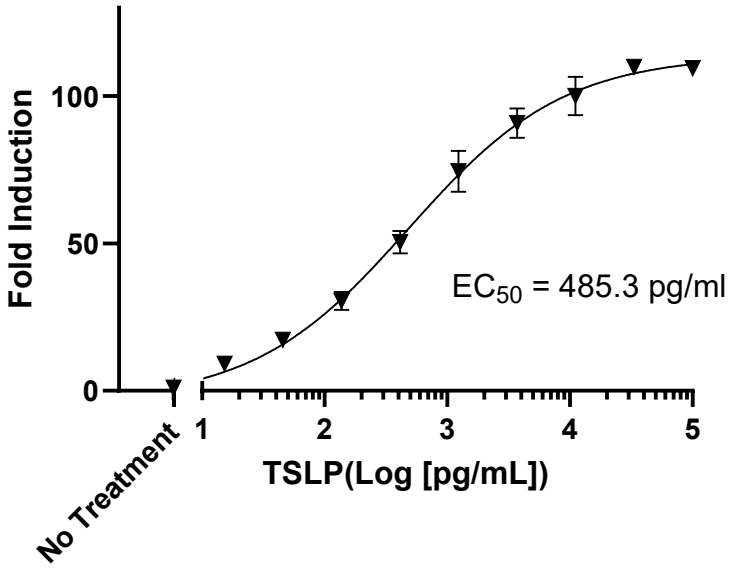

Figure 3: Dose-dependent response of TSLP Responsive Luciferase Reporter Ba/F3 Cell Line to human TSLP.
TSLP Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of recombinant human TSLP for 5-6 hours and luciferase activity was measured using the One-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of TSLP stimulated cells against the activity of cells without TSLP (unstimulated).

B. Inhibition of TSLP stimulation by anti-TSLP antibodies

- This experiment measures the effect of compounds, such as neutralizing antibodies, against stimulation by TSLP.
- The assay should include "Stimulated, No Compound", "Unstimulated, No Compound", "Background Luminescence" and "Test Compound" conditions.

1. Count cells and determine the cell density.
    a. If the cell density is <1.5 x 10^6 cells/ml, proceed with assay as below.
    b. If the cell density is >1.5 x 10^6 cells/ml, dilute cells in fresh Growth Media 8C.

    **Note: it is critical to start with a healthy cell population for this assay. Beginning with over-confluent cells will substantially reduce the signal window for the assay.*

2. Collect cells from culture and wash cells once with Thaw Medium 8.

3. Seed cells at a density of 25,000 cells/well in 80 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Leave a few empty wells as "Background Luminescence" control.

4. Incubate at 37°C with 5% CO_2 for ~40 hours.

   Note: It is critical to include a ~40-hour starvation in Thaw Media without mIL3 to reduce background signal (as mIL3 also stimulates the STAT5 luciferase reporter).

5. Prepare a 50 ng/ml solution of human TSLP in Thaw Medium 8 (10-fold the final desired concentration of 5 ng/ml).

6. Prepare a 3-fold increment serial dilution of anti-TSLP antibody in Thaw Medium 8 at concentrations 10-fold higher than the desired final concentrations.

7. Prepare a separate 96 well plate for pre-incubations. Add 10 µl/well of each dilution of anti-TSLP antibody prepared in step 6 and 10 µl/well of TSLP prepared in step 5. This is the TSLP/Antibody Mix. Pre-incubate at room temperature for 30 to 60 minutes.

8. Add 20 µl of each TSLP/Antibody Mix dilution to the "Test Compounds" wells.

9. Add 20 µl of 50 ng/ml TSLP Mix to the "Stimulated, No Compound".

10. Add 20 µl of Thaw Medium 8 to the "Unstimulated, No Compound" wells.

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

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

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

14. Incubate at RT for ~15 minutes.

15. Measure luminescence using a luminometer.

16. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The Percent Luminescence of STAT5 luciferase reporter expression is the background-subtracted luminescence of compound-treated stimulated cells divided by the background-subtracted luminescence of unstimulated control wells.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ compound\ treated\ cells - background}{luminescence\ of\ unstimulated, no\ compound\ treated\ cells - background}\right) x100$$

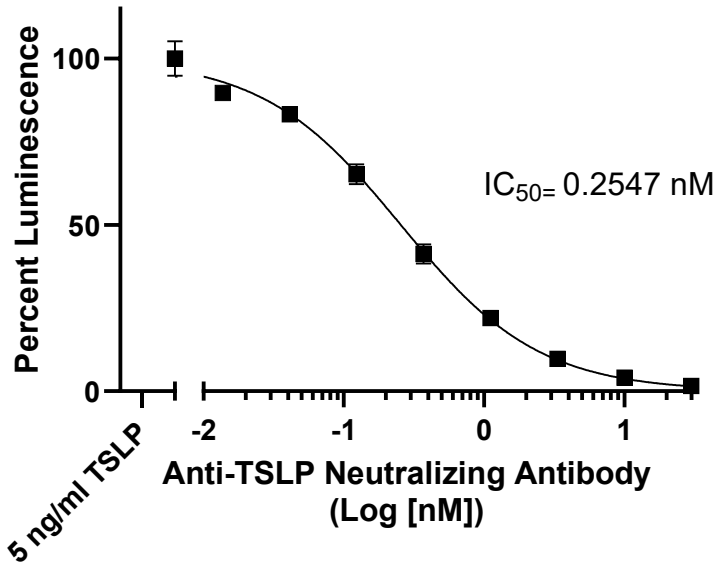

Figure 4: Dose-dependent response of TSLP Responsive Luciferase Reporter Ba/F3 Cell Line to Anti-TSLP Neutralizing Antibody.
A serial dilution of Anti-TSLP Neutralizing Antibody (#102138) was prepared and pre-incubated with TSLP for 30 minutes. After the 30-minute pre-incubation, the anti-TSLP + TSLP mixture was added to the cells and incubated for 5 hours. Luciferase activity was measured using One-Step™ luciferase Assay System. Results are shown as percentage of STAT5 reporter activity (compared to cells stimulated by TSLP in the absence of antibody, set at 100%).

C. Inhibition of TSLP stimulation by JAK 1/2 Inhibitors

- This experiment measures the effect of compounds, such as JAK inhibitors, against stimulation by TSLP.
- The assay should include "Stimulated, No Compound", "Unstimulated, No Compound", "Background Luminescence" and "Test Compound" conditions.

1. Count cells and determine the cell density.
    a. If the cell density is <1.5 x 10^6 cells/ml, proceed with assay as below.
    b. If the cell density is >1.5 x 10^6 cells/ml, dilute cells in fresh Growth Media 8C.

    **Note: it is critical to start with a healthy cell population for this assay. Beginning with over-confluent cells will substantially reduce the signal window for the assay.*

2. Collect cells from culture and wash cells once with Thaw Medium 8.

3. Seed cells at a density of 25,000 cells/well in 80 µl of Thaw Medium 8 into a white, clear-bottom 96-well cell culture plate. Leave a few empty wells as "Background Luminescence" control.

4. Incubate at 37°C with 5% CO_2 for ~40 hours.

Note: It is critical to include a ~40-hour starvation in Thaw Media without mIL3 to reduce background signal (as mIL3 also stimulates the STAT5 luciferase reporter).

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

6. Add 10 µl of each dilution to the "Test Compound" wells.

7. Add 10 µl of Thaw Medium 8 to the "Stimulated, No Compound" and "Unstimulated, No Compound" wells.

8. Incubate at 37°C with 5% CO_2 for 30 to 60 minutes.

9. Prepare a 50 ng/ml solution of human TSLP in Thaw Medium 8 (10-fold the final desired concentration of 5 ng/ml) (10 µl/well).

10. Add 10 µl of diluted human TSLP to the ""Test Compound" and "Stimulated, No Compound" wells.

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

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

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

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

15. Incubate at RT for ~15 minutes.

16. Measure luminescence using a luminometer.

17. Data Analysis: Subtract the background luminescence from the luminescence reading of all the wells. The Percent Luminescence of STAT5 luciferase reporter expression is the background-subtracted luminescence of compound-treated stimulated cells divided by the background-subtracted luminescence of unstimulated control wells.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ compound\ treated\ cells - background}{luminescence\ of\ unstimulated, no\ compound\ treated\ cells - background}\right) x100$$

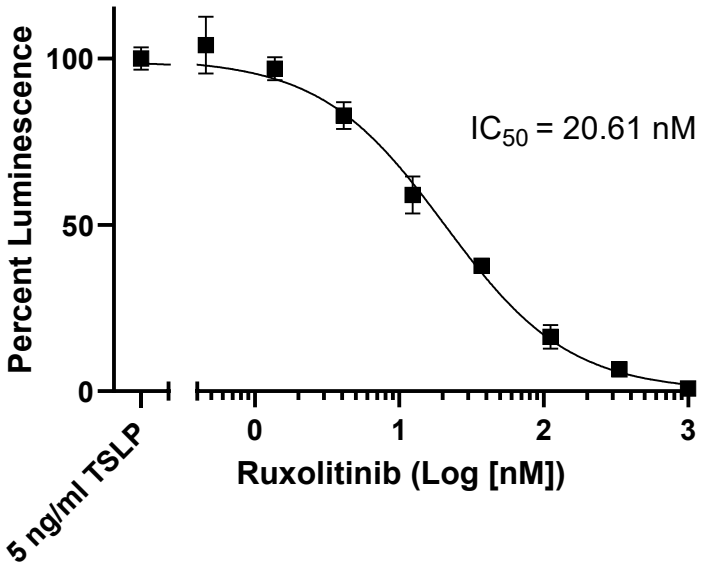

Figure 5: Dose-dependent response of TSLP Responsive Luciferase Reporter Ba/F3 Cell Line to the JAK 1/2 Inhibitor Ruxolitinib.
TSLP Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of Ruxolitinib for 30 to 60 minutes before stimulation with 5 ng/ml of TSLP. Luciferase activity was measured using One-Step™ luciferase Assay System. Results are shown as percentage of STAT5 reporter activity (compared to cells stimulated by TSLP in the absence of antibody, set at 100%).

Data shown is representative.

References

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Marković I. and Savvides S., 2020 *Front. Immunol.,* 11.
Palacios R. *et al.,* 1984 *Nature* 309 (5964): 126-131.
Rochman Y., Spolski R. and Leonard, W., 2009 *Nat Rev Immunol,* 9: 480–490.
Verstraete K. *et al.,* 2014 *Nat Struct Mol Biol*, 21: 375–382.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
TSLP Responsive Luciferase Reporter U937 Cell Line	82501	2 vials
STAT5 Luciferase Reporter Ba/F3 Cell Line	79722	2 vials
Human Thymic Stromal Lymphopoietin Recombinant	90250	2 µg/10 µg
STAT5 Luciferase Reporter Lentivirus	79745	500 µl x 2
STAT5 Peptide	79864	500 µg
STAT5 Luciferase Reporter U937 Cell Line	79941	2 vials

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