SZABO SCANDIC

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# Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

## Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

## Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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**Description**

The IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line is a murine Ba/F3 cell line engineered to express both human IL-5RA (IL-5 receptor alpha, also known as CD125) (NM_175726.4) and human CSF2RB (common receptor beta chain also known as IL5RB or CD131) (NM_000395.3), separated by a self-cleaving P2A peptide. The construct was delivered by lentiviral transduction of the STAT5 Luciferase Reporter Ba/F3 Cell Line (#79772), which expresses a firefly luciferase reporter driven by the STAT5 (signal transducer and activator of transcription 5) response elements located upstream of the minimal TATA promoter. After activation by IL-5, 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 IL-5. Additional functional validation demonstrates that IL-5-induced luciferase activity can be inhibited by either anti-IL-5 or anti-IL-5R neutralizing antibodies.

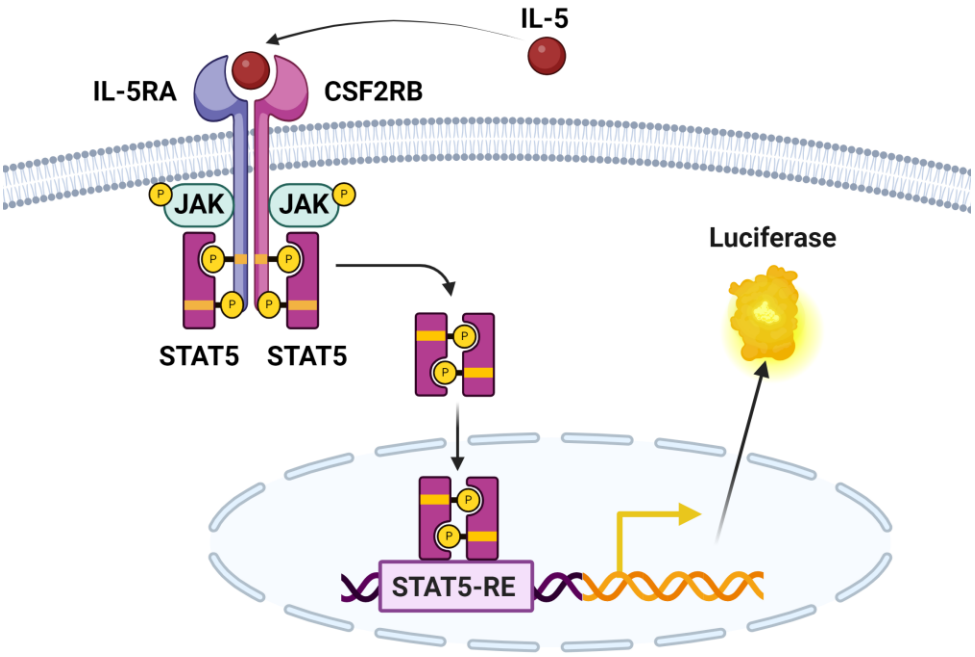


*Figure 1: Illustration of the mechanism of IL-5 Luciferase Reporter Ba/F3 Cell Line.*
IL-5 binds to IL-5 receptor alpha subunit, facilitating its interaction with the common receptor beta chain (CSF2RB), and activating downstream JAK1/2 (Janus kinase 1/2) tyrosine kinases, which phosphorylate the transcription factor STAT5 (signal transducer and activator of transcription 5). 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**

IL-5 (interleukin-5) is a pro-inflammatory protein that functions as an intermediary cytokine in the $T_H2$ (T helper 2) cells response in asthma and other eosinophilic allergic diseases. IL-5 is produced by $T_H2$ cells and ILC2 (group 2 innate lymphoid cells) cells in response to stimulation by alarmins such as TSLP (thymic stromal lymphopoietin) or IL-33, which initiate the inflammatory cascade. IL-5 functions by promoting eosinophil maturation, activation, and migration into target tissues. It binds to the IL-5 specific cell surface receptor IL-5RA (IL-5 receptor alpha) and further recruits the common beta chain receptor (CSF2RB) to initiate downstream signaling through JAK1/2 (Janus kinase 1/2) and STAT5. Activated eosinophils contribute to airway damage through degranulation, resulting in the release of pro-inflammatory cytokines and enzymatic mediators contributing to local inflammation and tissue damage.

IL-5 signaling plays a critical role in allergic and eosinophilic asthma subtypes and has been an active area for drug discovery. Antibodies targeting both IL-5 ligand and IL-5 Receptor (IL-5RA) are FDA (Food and Drug Administration) approved for the treatment of severe eosinophilic asthma and other eosinophilic conditions. These antibodies function by neutralizing IL-5-mediated signaling in eosinophils. Additionally, IL-5RA targeting antibodies and CAR (chimeric antigen receptor)-T cells can induce immune-mediated cytotoxicity of IL-5RA-expressing cells, including eosinophils through ADCC (antibody-dependent cellular cytotoxicity) and CAR-T cell mediated killing respectively.

**Application**
Screen and characterize modulators of IL-5 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**
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 |
| Human IL-5 Recombinant | BPS Bioscience #102652 |
| Mepolizumab | BPS Bioscience #82842 |
| Anti IL-5RA Neutralizing Antibody | BPS Bioscience # 102485 |
| Benralizumab | BPS Bioscience #82843 |
| Ruxolitinib Phosphate | BPS Bioscience #27059 |
| 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 (#79652) with mouse IL-3 (#90189):*
RPMI 1640 medium supplemented with 10% heat-inactivated FBS, 1% Penicillin/Streptomycin and 5 ng/ml mouse IL-3 (#90189).

*Complete Growth Medium: Growth Medium 8C (#82505) with mouse IL-3 (#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 (#90189).

*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**

**Functional Validation**

- The following assays are designed for either 96-well or 384-well format, as specified. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

**A. Agonist evaluation (96-well)**

- 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.
- The assay should be performed in triplicate.

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 described 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 40,000 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.

5. Prepare a 3-fold increment serial dilution of human IL-5 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.

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

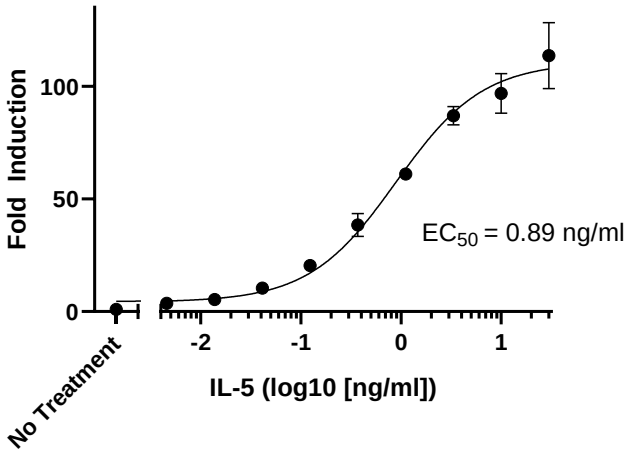


*Figure 2: Dose-dependent response of IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line to human IL-5.*
IL-5 Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of recombinant human IL-5 for 5-6 hours and luciferase activity was measured using the One-Step™ Luciferase Assay System. The results are shown as fold induction of luciferase reporter expression. Fold induction was determined by comparing luciferase activity of IL-5 stimulated cells versus of the activity of cells without IL-5 (unstimulated).

**B. Agonist evaluation (384-well)**

- 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.
- The assay should be performed in quadruplicate.

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 described 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 10,000 cells/well in 20 µl of Thaw Medium 8 into a white, clear-bottom 384-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.

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

6. Add 5 µl of each dilution to "Stimulated" wells.

7. Add 5 µl of Thaw Medium 8 to "Unstimulated" wells.

8. Add 25 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 25 µl/well of ONE-Step™ Luciferase reagent.

11. Incubate at 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.

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

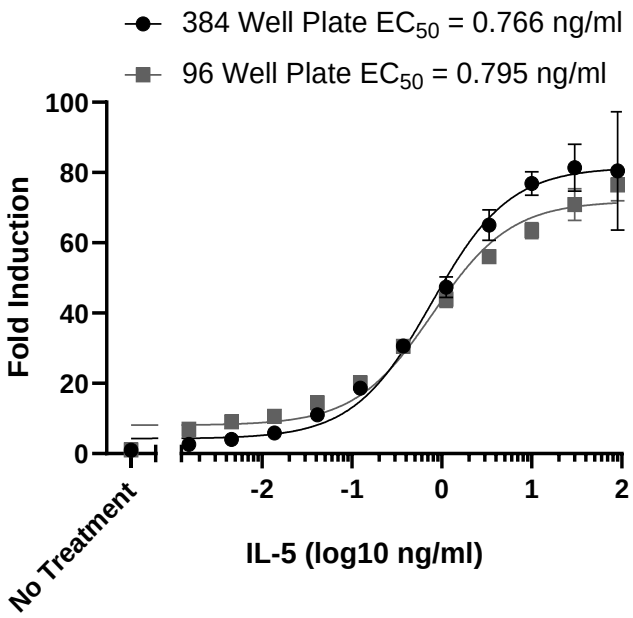


*Figure 3: Dose-dependent response of IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line to human IL-5 in 96 well and 384 well formats.*
IL-5 Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of recombinant human IL-5 for 5-6 hours and luciferase activity was measured 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 IL-5 stimulated cells versus the activity of cells without IL-5 (unstimulated).

**C. Inhibition of IL-5 stimulation by anti-IL-5 antibodies**

- This experiment measures the effect of compounds, such as neutralizing antibodies, against stimulation by IL-5.
- The assay should include "Stimulated, No Compound", "Unstimulated, No Compound", "Background Luminescence" and "Stimulated, Test Compound" conditions.
- The assay should be performed in triplicate.

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 described 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 40,000 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.

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

6. Prepare a 3-fold increment serial dilution of anti-IL-5 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-IL-5 antibody prepared in step 6 and 10 µl/well of IL-5 prepared in step 5. This is the IL-5 /Antibody Mix. Pre-incubate at RT for 30 to 60 minutes.

8. Add 20 µl of each IL-5/Antibody Mix dilution to the "Stimulated, Test Compounds" wells.

9. Add 20 µl of the 50 ng/ml IL-5 solution 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 stimulated, no compound control wells.

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

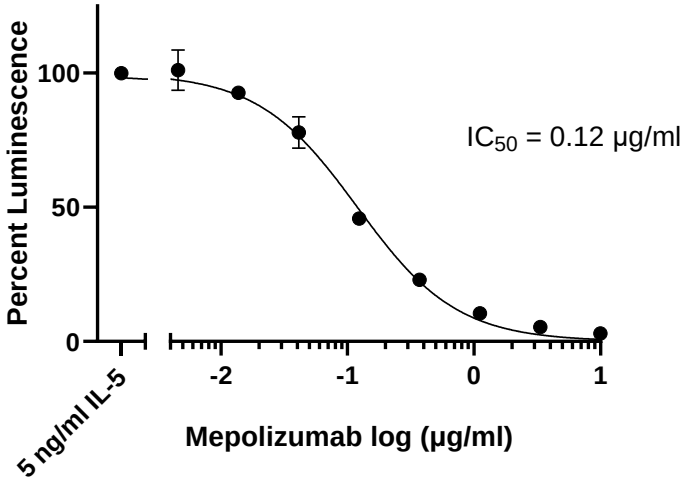


*Figure 4: Dose-dependent response of IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line to Mepolizumab.*
A serial dilution of Mepolizumab (#82842) was prepared and pre-incubated with IL-5 for 30 minutes. After the 30-minute pre-incubation, the IL-5/ Antibody Mix was added to the cells. After a 5-hour incubation, luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of STAT5 reporter activity (compared to cells stimulated by IL-5 in the absence of antibody, set at 100%).

**D. Inhibition of IL-5 stimulation by JAK 1/2 Inhibitors or anti-IL-5RA Antibodies**

- This experiment measures the effect of compounds, such as JAK inhibitors or Anti-IL-5 Receptor antibodies, against stimulation by IL-5.
- The assay should include "Stimulated, No Compound", "Unstimulated, No Compound", "Background Luminescence" and "Stimulated, Test Compound" conditions.
- The assay should be performed in triplicate.

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 described 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 40,000 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.

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 "Stimulated, 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 IL-5 in Thaw Medium 8 (10-fold the final desired concentration of 5 ng/ml) (10 µl/well).

10. Add 10 µl of diluted human IL-5 to the "Stimulated, 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 stimulated, no compound control wells.

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

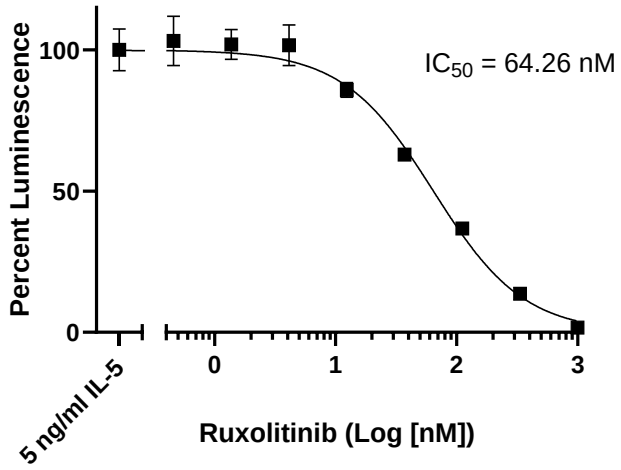


*Figure 5: Dose-dependent response of IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line to the JAK 1/2 Inhibitor Ruxolitinib.*

IL-5 Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of Ruxolitinib (#27059) for 30 minutes before stimulation with 5 ng/ml of IL-5. Luciferase activity was measured using One-Step™ luciferase Assay System. Results are shown as percentage of STAT5 reporter activity (compared to cells stimulated by IL-5 in the absence of inhibitor, set at 100%).

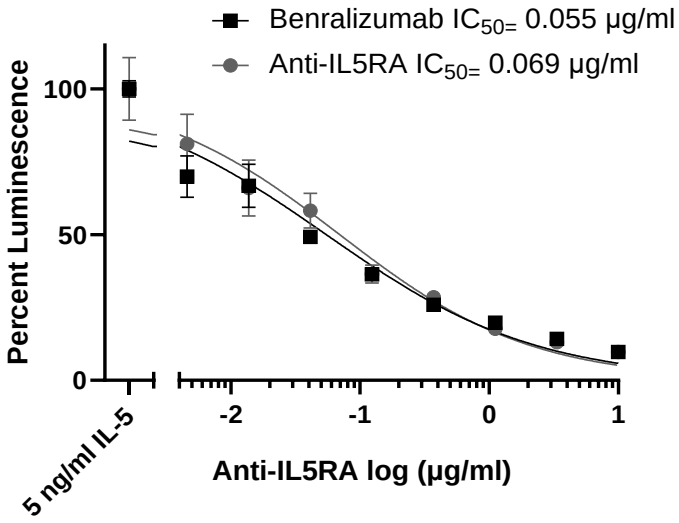


*Figure 6: Dose-dependent response of IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line to Anti-IL-5RA Antibodies.*

IL-5 Responsive Luciferase Reporter Ba/F3 cells were incubated with increasing concentrations of Anti-IL-5RA antibody (#102485) or Benralizumab (#82843) for 30 minutes before stimulation with 5 ng/ml of IL-5. Luciferase activity was measured using One-Step™ luciferase Assay System. Results are shown as percentage of STAT5 reporter activity (compared to cells stimulated by IL-5 in the absence of antibody, set at 100%).

*Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

**References**

Kim J-E., *et al*., 2021 *Front. Immunol.* 11:593748.
Lawrence M., *et al.,* 2019 *Ann of Allergy, Asthma Immunol* 120(4): 376 – 381.
Matucci A., *et al.,* 2019 *Respir Med* 160:105819.
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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 Ba/F3 Cell Line | 79722 | 2 vials |
| TSLP Responsive Ba/F3 Cell Line | 82500 | 2 vials |
| IL-33 Responsive Jurkat Cell Line | 82800 | 2 vials |
| STAT5 Luciferase Reporter Lentivirus | 79745 | 500 µl x 2 |
| STAT5 Peptide | 79864 | 500 µg |
| STAT5 Luciferase Reporter U937 Cell Line | 79941 | 2 vials |

*Version 013025*