IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line

#78941

Description

IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line is a HEK293 cell line with stably integrated Firefly luciferase reporter under the control of STAT6 response element and human STAT6 (signal transduced and activator of transcription factor 6), resulting in a fully functional STAT6 signaling pathway.

This cell line has been validated with activation assays with human IL-4 (interleukin-4) and IL-13 (interleukin-13) and the use of anti-IL-4R and anti-IL-13R antagonists.

Background

The transcription factor STAT6 (signal transduced and activator of transcription factor 6) plays a vitally important role in immune cells. IL-4-activated STAT6 signaling is required for Th2 differentiation and immunoglobulin class switching and it also mediates direct repression of inflammatory enhancers in macrophages. STAT6 is involved in several aspects of allergic inflammatory disease, like airway hyperresponsiveness, eosinophilic infiltration, and mast cells responses. STAT6 is activated primarily by IL-4 and the related IL-13, with have overlapping biologic profiles. Upon IL-4 and IL-13 binding, the receptor complex composed of IL-4Ralpha and IL-13Ralpha1 activates the receptor-associated Janus kinases (JAK1 and Tyk2), leading to the activation of STAT6. Activated STAT6 forms homodimers that translocate to the nucleus where they bind the promoter of responsive genes, inducing gene transcription. While both cytokines activate STAT6 signal, it has been proven that IL-4 is more potent in engaging this signaling pathway than IL-13. Dysfunction of the STAT6 pathway leads to allergic reactions (asthma, atopic dermatitis, food allergies) and cancer (Hodgkin, central nervous system, and follicular lymphoma). STAT6 can serve as a diagnostic and prognostic tool. Inhibitors have been shown to reduce acute inflammation in animal models, and future developments may lead to new therapeutical tools for STAT6 related pathologies.

Application

- Screen and characterize activators or inhibitors of the STAT6 signaling pathway.
- Screening anti-IL-4, anti-IL-4R, anti-IL-13, or anti-IL-13R antibodies.

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

HEK293, Human Embryonic Kidney, epithelial-like cells, adherent

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 1	BPS Bioscience #60187
Growth Medium 1M	BPS Bioscience #79723

Materials Used in Cellular Assay

Name	Ordering Information
Thaw Medium 1	BPS Bioscience #60187
Human Interleukin-4	BPS Bioscience #90193
Human Interleukin-13	BPS Bioscience #90179
Dupilumab Recombinant Human Monoclonal Antibody	Invitrogen #MA5-41985
Lebrikizumab	BPS Bioscience #82692
Anti-IL-13 Neutralizing Antibody	BPS Bioscience #102208
AS1517499	BPS Bioscience #82693
Clear-bottom, white 96-well tissue culture-treated plate	Corning #3610
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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 and Functional Cellular Assay

Thaw Medium 1 (BPS Bioscience #60187):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

Growth Medium 1M (BPS Bioscience #79723):

MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin, 400 µg/ml of Geneticin and 0.5 µg/ml Puromycin.

Cell Culture Protocol

Note: HEK293 cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 1.
   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 Thaw Medium 1.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 1 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 are fully confluent. At first passage and subsequent passages, use Growth Medium 1M.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1M and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 1M.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 weekly or twice per week.

   Note: Just after thawing and when cells are at low density, the cells may grow at a slower rate. It is recommended to split the cells at a 1/4 ratio in those cases. After several passages, the cell growth rate increases, and the cells can be split using a higher ratio.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+} and detach the cells from the culture vessel with 0.05% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 1M and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. 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.

5. 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 were designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.

A. Dose response of hIL-4 and hIL-13 in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line

- This experiment measures the effect of a compound on reporter activation.
- All conditions should be performed in triplicate.
- The assay should include "Stimulated", "Cell-Free Control" and "Unstimulated Control" conditions.

1. Seed IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 cells at a density of ~20,000 cells/well in 100 µl of Thaw Medium 1 into a clear-bottom, white 96-well plate. Leave a few empty wells as "Cell-Free Control" wells (luminescence background control).

2. Incubate the plate at 37°C in a 5% CO_2 incubator for 24 hours.

3. Prepare the compounds of interest at the concentration to be tested, in Thaw Medium 1 (100 µl/well).

4. Carefully remove the cell culture medium from all wells.

5. Add 100 µl of the compounds to be tested to the "Stimulated" wells.

6. Add 100 µl of Thaw Medium 1 to the "Unstimulated Control" (to determine the unstimulated luminescence from IL-4/IL-13 Responsive STAT6 Luciferase reporter HEK293 cells) and "Cell-Free Control" wells.

7. Incubate the plate at 37°C with 5% CO_2 for ~6 hours.

8. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at room temperature for ~15 to 30 minutes.

9. Measure luminescence using a luminometer.

10. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The fold induction of STAT6 luciferase reporter expression is the average background-subtracted luminescence of stimulated wells divided by the average background-subtracted luminescence of unstimulated control wells.

$$Fold\ induction = \frac{Luminescence\ of\ \ Stimulated\ Wells - avg.background}{Avg.Luminescence\ of\ Unstimulated\ Wells - avg.background}$$

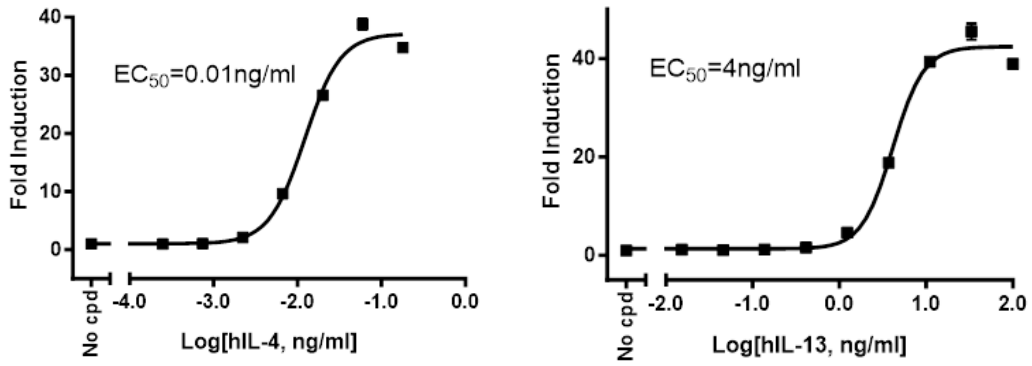

Figure 1: Reporter activation of IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line in response to human IL-4 and IL-13.
IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 cells were incubated with increasing concentrations of human IL-4 (left panel) and IL-13 (right panel) for 6 hours. Luciferase activity was measured using the ONE-Step™ Luciferase Assay System. The results are shown as fold induction of STAT6 luciferase reporter expression in relation to the activity of cells without agonist.

B. Inhibition of human IL-4-induced STAT6 activity by anti-human IL-4R antibody in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.

- The effect of an antagonist compound is measured against agonist activation.
- All conditions should be performed in triplicate.
- The assay should include “Stimulated, No Antagonist”, “Unstimulated, No Antagonist”, “Cell-Free Control”, and “Antagonist” conditions.

1. Seed IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 cells at a density of 20,000 cells/well in 100 µl of Thaw Medium 1, into a white clear-bottom 96-well cell culture plate. Keep wells without cells as “Cell-Free Control” (for determining the background luminescence).

2. Incubate the cells at 37°C with 5% CO_2 overnight.

3. Prepare a three-fold serial dilution of anti-IL-4R antagonist antibody in Thaw Medium 1 (50 µl/well) at concentrations 2-fold higher than the desired final concentrations.

4. Remove the cell culture medium from the cells.

5. Add 50 µl of each dilution of antagonist antibody to the “Antagonist” wells.

6. Add 50 µl of Thaw Medium 1 to the “Stimulated, No Antagonist” and “Unstimulated, No Antagonist” wells.

7. Incubate the cells at 37°C in 5% CO_2 for 1-2 hours.

8. Prepare hIL-4 in Thaw Medium 1 at a concentration of 0.06 ng/ml (the final concentration will be 0.03 ng/ml) (50 µl/well).

9. Add 50 µl of hIL-4 to the "Antagonist" and "Stimulated, No Antagonist" wells.

10. Add 50 µl of Thaw Medium 1 to the "Unstimulated, No Antagonist" (for determining STAT6 basal activity) wells.

11. Add 100 µl of Thaw Medium 1 to "Cell-Free Control" wells.

12. Incubate at 37°C in 5% CO_2 for ~6 hours.

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

14. Incubate with gentle agitation at room temperature for ~15 to 30 minutes.

15. Measure luminescence using a luminometer.

16. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of antagonist-treated cells divided by the background-subtracted luminescence of agonist-activated control cells (Stimulated, No Antagonist) multiplied by 100. IL-4-stimulated cells in the absence of antagonist was set at 100%.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ Antagonist\ treated\ cells - avg.background}{luminescence\ of\ Stimulated,No\ Antagonist\ cells - avg.background}\right) \text{x}100$$

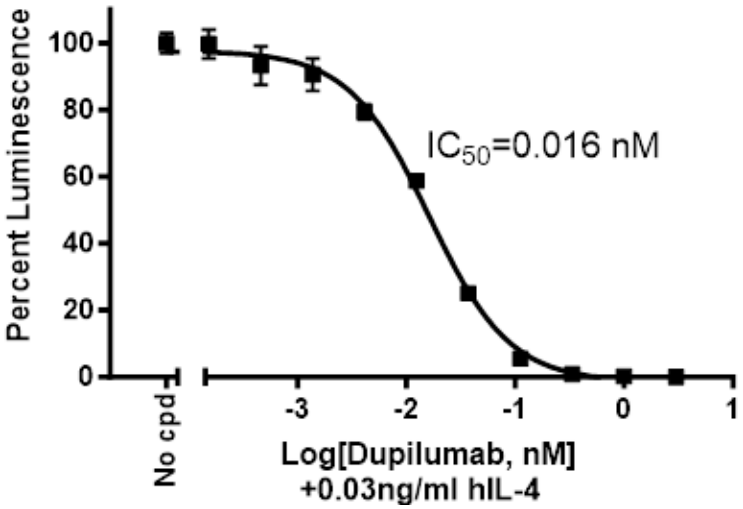

Figure 2. Inhibition of hIL-4-induced reporter activity by Dupilumab Recombinant Human Monoclonal Antibody in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.
Cells were incubated with increasing concentrations of the anti-IL4R antagonist, Dupilumab, for 2 hours, followed by IL-4 (0.03 ng/ml) for 6 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of STAT6 reporter activity (compared to cells stimulated by IL-4 without antagonist).

C. Inhibition of hIL-13-induced STAT6 activity by anti-human IL-13 antibody in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.

- The effect of an antagonist compound is measured against agonist activation.
- All conditions should be performed in triplicate.
- The assay should include "Stimulated, No Antagonist", "Unstimulated, No Antagonist", "Cell-Free Control", and "Antagonist" conditions.
- We recommend testing your lot of IL-13 in dose response prior to performing the inhibition assay. Choose an approximately EC_{80} concentration of IL-13 and run the inhibition assay as directed. For the data shown below, we used 30 ng/ml of recombinant human IL-13. Your optimal concentration may vary.

1. Seed IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 cells at a density of 20,000 cells/well in 100 µl of Thaw Medium 1, into a white clear-bottom 96-well cell culture plate. Keep wells without cells as "Cell-Free Control" (for determining the background luminescence).

2. Incubate the cells at 37°C with 5% CO_2 overnight.

3. Prepare a three-fold serial dilution of anti-IL-13 antagonist antibody in Thaw Medium 1 (50 µl/well) at concentrations 2-fold higher than the desired final concentrations.

4. Prepare hIL-13 in Thaw Medium 1 at a concentration 2-fold higher than the final desired concentration.

5. Combine 50 µl of hIL-13 with 50 µl of the dilutions of anti-IL-13 antibody and incubate at room temperature for 30 minutes.

6. Remove cell culture media and add 100 µl of each mix of hIL-13/anti-IL-13 antibody dilutions to the "Antagonist" wells.

7. Remove cell culture media and add 100 µl of Thaw Medium 1 to the "Unstimulated, No Antagonist" wells.

8. Remove cell culture and add 50 µl of hIL-13 and 50 µl of Thaw Medium 1 to the "Stimulated, No Antagonist" wells.

9. Add 100 µl of Thaw Medium 1 to "Cell-Free Control" wells.

10. Incubate at 37°C in 5% CO_2 for ~6 hours.

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

12. Incubate with gentle agitation at room temperature for ~15 to 30 minutes.

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of Antagonist-treated cells divided by the background-subtracted luminescence of agonist-activated control cells (Stimulated, No Antagonist), multiplied by 100. IL-13-stimulated cells in the absence of antagonist was set at 100%.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ Antagonist\ treated\ cells - avg.background}{luminescence\ of\ Stimulated, No\ Antagonist cells - avg.background}\right) x100$$

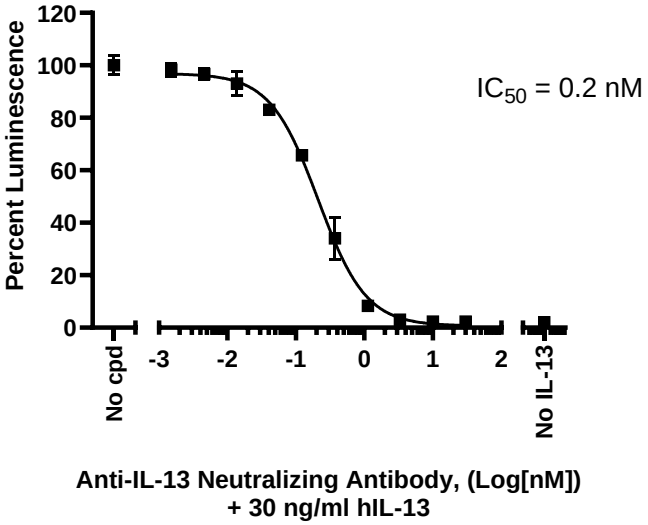

Figure 3. Inhibition of hIL-13-induced reporter activity by Anti-IL-13 Neutralizing Antibody in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.

Diluted hIL-13 (30 ng/ml) was incubated with increasing concentrations of the Anti-IL-13 Neutralizing Antibody for 30 minutes, and then added to cells for 6 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of STAT6 reporter activity (compared to cells stimulated by IL-13 without antagonist).

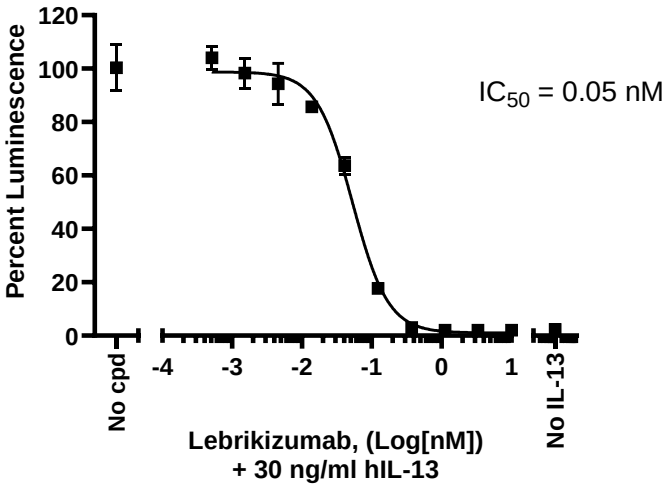

Figure 4. Inhibition of hIL-13-induced reporter activity by Lebrikizumab in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.

Diluted IL-13 (30 ng/ml) was incubated with increasing concentrations of the anti-IL-13 antagonist, Lebrikizumab, for 30 minutes, and then added to cells for 6 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of STAT6 reporter activity (compared to cells stimulated by IL-13 and without antagonist).

D. Inhibition of cytokine-induced STAT6 activity by small molecule inhibitors in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.

- All conditions should be performed in triplicate.
- The assay should include "Stimulated, No Inhibitor", "Unstimulated, No Inhibitor", "Cell-Free Control", and "Inhibitor" conditions.

1. Seed IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 cells at a density of 20,000 cells/well in 100 µl of Thaw Medium 1, into a white clear-bottom 96-well cell culture plate. Keep wells without cells as "Cell-Free Control" (for determining the background luminescence).

2. Incubate the cells at 37°C with 5% CO_2 overnight.

3. Prepare a three-fold serial dilution of the STAT6 inhibitor AS1517499 in Thaw Medium 1 (50 µl/well) at concentrations 2-fold higher than the desired final concentrations.

4. Remove the cell culture medium from the cells.

5. Add 50 µl of each dilution of inhibitor to "Inhibitor" wells.

6. Add 50 µl of Thaw Medium 1 to the "Stimulated, No Inhibitor" and "Unstimulated, No Inhibitor" wells.

7. Incubate the cells at 37°C in 5% CO_2 for 1 hour.

8. Prepare cytokine solutions in Thaw Medium 1 at concentrations 2-fold higher than the desired final concentrations (final [hIL-4] = 0.03 ng/ml, final [hIL-13] = 30 ng/ml).

9. Add 50 µl of desired cytokine to the "Inhibitor" and "Stimulated, No Inhibitor" wells.

10. Add 50 µl of Thaw Medium 1 to the "Unstimulated, No Inhibitor" (for determining STAT6 basal activity) wells.

11. Add 100 µl of Thaw Medium 1 to "Cell-Free Control" wells.

12. Incubate at 37°C in 5% CO_2 for ~6 hours.

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

14. Incubate with gentle agitation at room temperature for ~15 to 30 minutes.

15. Measure luminescence using a luminometer.

16. Data Analysis: Subtract the background luminescence from the luminescence reading of all conditions. The percent luminescence is the background-subtracted luminescence of inhibitor-treated cells divided by the background-subtracted luminescence of agonist-activated control cells (Stimulated, No Inhibitor) multiplied by 100. Agonist-stimulated cells in the absence of inhibitor was set at 100%.

$$Percent\ Luminescence = \left(\frac{luminescence\ of\ Inhibitor\ treated\ cells - avg.background}{luminescence\ of\ Stimulated,\ \ No\ Inhibitor\ cells - avg.background}\right) x100$$

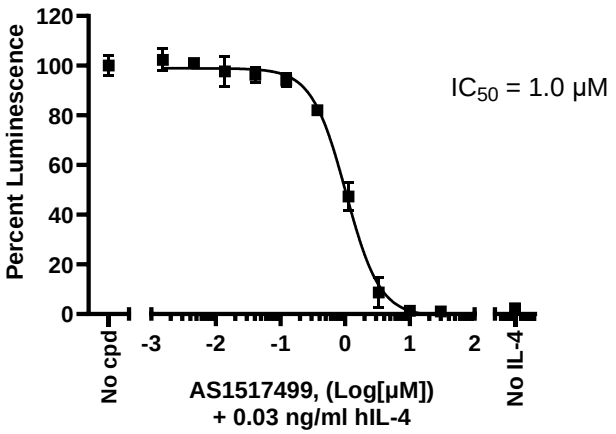

Figure 5. Inhibition of hIL-4-induced reporter activity by AS1517499 in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.
Cells were incubated with increasing concentrations of AS1517499 for 1 hour, followed by IL-4 (0.03 ng/ml) for 6 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of STAT6 reporter activity (compared to cells stimulated by IL-4 without inhibitor).

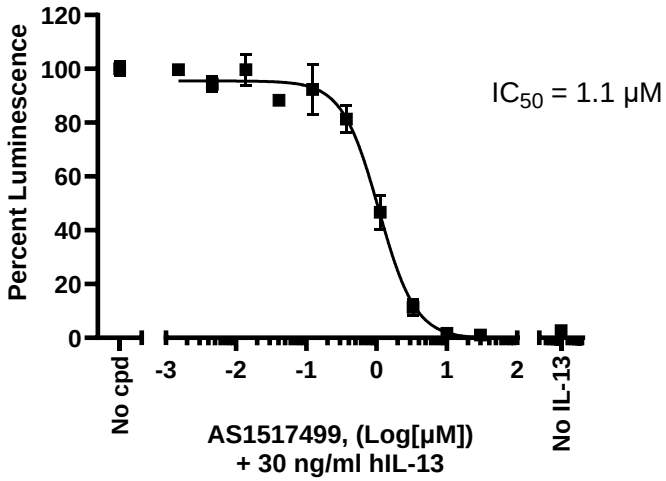

Figure 6. Inhibition of hIL-13-induced reporter activity by AS1517499 in IL-4/IL-13 Responsive STAT6 Luciferase Reporter HEK293 Cell Line.
Cells were incubated with increasing concentrations of AS1517499 for 1 hour, followed by IL-13 (30 ng/ml) for 6 hours. Luciferase activity was measured using One-Step™ Luciferase Assay System. Results are shown as percentage of STAT6 reporter activity (compared to cells stimulated by IL-13 without inhibitor).

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

References

Kasaian MT., *et al.*, 2013. *Am J Respir Cell Mol Biol.* 49(1):37-46.
Czimmerer Z., *et al.*, 2018. *Immunity* 48(1):75-90.e6.

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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*
STAT6 Luciferase Reporter Lentivirus (STAT6 Signaling Pathway)	78799	500 µl x 2
STAT3 Luciferase Reporter HEK293 Cell Line	79800-P	2 vials
ZsGreen/Luciferase Safe-Harbor HEK293 Cell Line	82091	2 vials

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