Description

The TGFβ/Activin A/Myostatin-Responsive Reporter HEK293 Cell Line is a HEK293 cell line expressing the Firefly luciferase reporter under the control of SMAD-responsive elements (SMAD binding elements, SBE). This cell line monitors the activity of the TGFβ (transforming growth factor beta)/SMAD signaling pathway.

This cell line has been validated by activation in response to human TGFβ1 and to other cytokines of the TGFβ1 superfamily such as Activin A, GDF-8/Myostatin, and GDF-11/BMP-11. Luciferase activity induced by human TGFβ1 and Activin A was decreased by SB525334, an inhibitor of the TGFβ1-receptor. Luciferase activity induced by Activin A, GDF-8/Myostatin, and GDF-11/BMP-11 was decreased by Bimagrumab, an antibody of the ActRII. Activin A induced luciferase activity was also decreased by Activin Blocker, an ActRIIA-Fc fusion protein, and an Anti-TGFβ antibody.

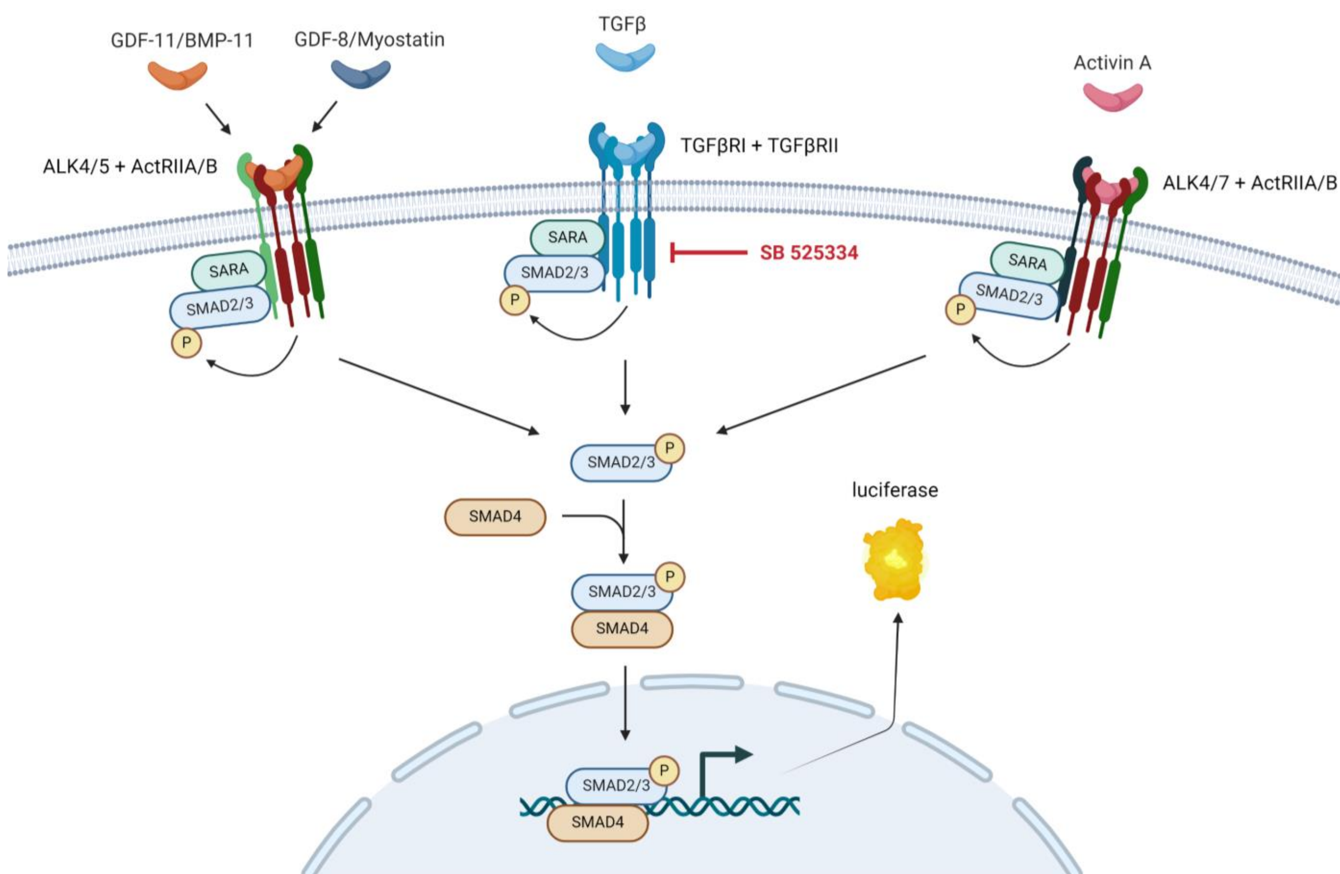

Figure 1: illustration of mechanism of action of the TGFβ/Activin A/Myostatin-Responsive Reporter HEK293 Cell Line.

(Note: the endogenous expression level of each of the receptors is dependent on the cell line. HEK293 cells have been described to express low levels of ALK7).

Background

The TGFβ signaling pathway participates in diverse cellular processes such as growth and differentiation, cell cycle arrest, and immune regulation. TGFβ signaling has been linked to heart disease, cancer, Alzheimer's disease, among others. TGFβ proteins bind to receptors on the cell surface, initiating a signaling cascade that leads to phosphorylation and activation of the signaling proteins SMAD2 (mothers against decapentaplegic homolog 2) and SMAD3, which then form a complex with SMAD4. The SMAD complex gets translocated to the nucleus and

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binds to the SMAD binding element (SBE) in the promoter of target genes, leading to transcription and expression of TGFβ/ SMAD responsive genes. The understanding and regulation of this pathway can provide essential clues for the development of therapeutical approaches for cancer treatment.

Application

- Monitor activation of the TGFβ signaling pathway.
- Screen for compounds that regulate the TGFβ/SMAD signaling pathway.

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 1B	BPS Bioscience #79531

Materials Used in Cellular Assay

Name	Ordering Information
Assay Medium 1B	BPS Bioscience #79617
Human TGFβ1	BPS Bioscience #90900
Recombinant Human/Murine/Rat Activin A	PeproTech #120-14E
Recombinant Human/Mouse/Rat GDF-8/Myostatin Protein	R&D Systems #788-G8
Recombinant Human/Mouse/Rat GDF-11/BMP-11 Protein	R&D Systems #1958-GD
Inhibitor SB525334	Selleckchem #356559-20-1
Bimagrumab	BPS Bioscience #82876
Activin Blocker	BPS Bioscience #102121
Anti-TGFβ Antibody	BPS Bioscience #102330
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

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 1B (BPS Bioscience #79531):
MEM medium supplemented with 10% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin plus 400 µg/ml of Geneticin.

Media Required for Functional Cellular Assay

Assay Medium 1B (BPS Bioscience #79617):
MEM Medium, 0.5% FBS, 1% non-essential amino acids, 1 mM Na pyruvate, 1% Penicillin/Streptomycin.

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 1B.

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 1B 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 1B.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:5 to 1:10 once or twice a 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 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 1B 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 ~2×10^6 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.
- Dose-response of agonist and antagonist is best performed using 3-fold serial dilutions.

A. Reporter activation by cytokines

- This experiment measures the effect of an agonist on reporter activation.
- All conditions should be performed in triplicate.
- The assay should include “Stimulated Cells”, with “Luminescence Background Control” and “Unstimulated Control” conditions.

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1. Seed TGFβ/Activin A/Moystatin-Responsive Luciferase Reporter HEK293 cells at a density of ~35,000 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. 24 hours later, carefully remove the medium from all wells and replace it with 90 µl of fresh Assay Medium 1B.

4. Incubate cells at 37°C in a 5% CO_2 incubator for ~4-5 hours.

5. Prepare serial dilutions of the agonist in Assay Medium 1B at concentrations 10-fold higher than the desired final concentrations.

6. Add 10 µl of each agonist dilution to the wells labeled as “Stimulated Cells”.

7. Add 10 µl of Assay Medium 1B to the wells labeled as “Unstimulated Control”.

8. Add 100 µl of Assay Medium 1B to the wells not containing cells and labeled as “Luminescence Background Control”.

9. Incubate the plate at 37°C in a CO_2 incubator overnight (~18 hours).

10. Add 100 µl of ONE-Step™ Luciferase reagent to each well and rock at Room Temperature (RT) for ~15 to 30 minutes.

11. Measure luminescence using a luminometer.

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

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

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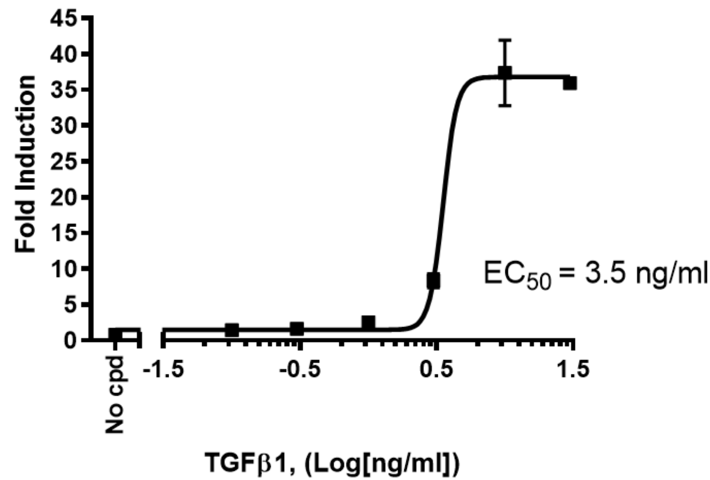

Figure 2: Reporter activation in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by the agonist TGFβ1.

TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of human TGFβ1 (#90900) for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

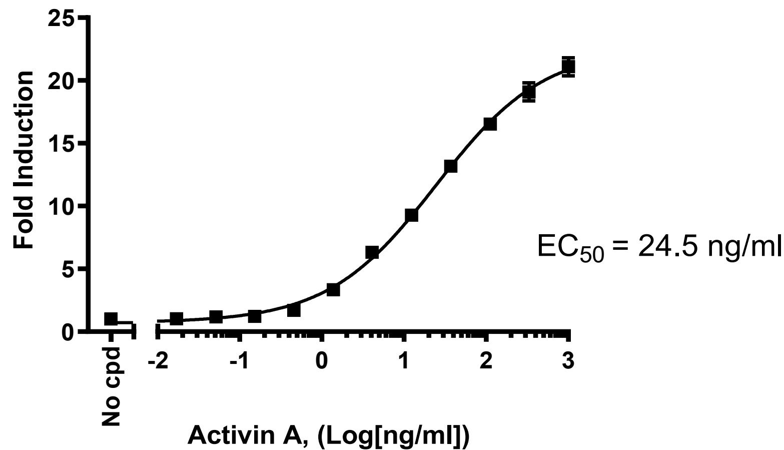

Figure 3: Reporter activation in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by the agonist Activin A.

TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of Activin A (PeproTech #120-14E) for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (BPS Bioscience #60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

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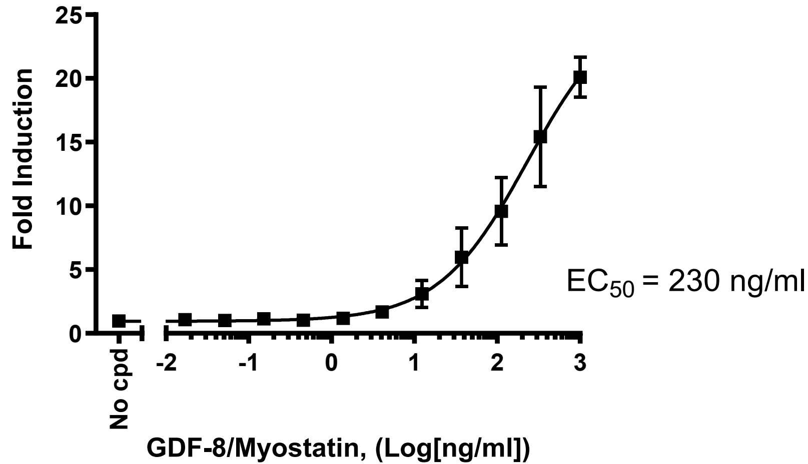

Figure 4: Reporter activation in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by the agonist GDF-8/Myostatin.
TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of GDF-8/Myostatin for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

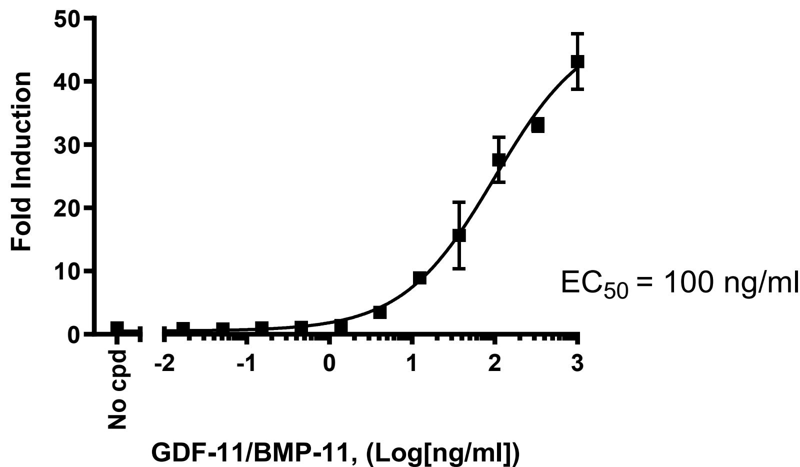

Figure 5: Reporter activation in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by the agonist GDF-11/BMP-11.
TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells were incubated with increasing concentrations of GDF-11/BMP-11 for 18 hours. Luciferase activity was measured using ONE-Step™ Luciferase Assay System (#60690). The results are shown as fold induction of SBE luciferase reporter expression in relation to the activity of cells in the absence of agonist.

B. Inhibition of agonist-induced reporter activity in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by an inhibitor of the TGFβ signaling pathway

- This experiment measures the effect of inhibitors of the TGFβ signaling pathway on agonist induced SBE reporter activation.
- All conditions should be performed in triplicate.
- The assay should include "Luminescence Background Control", "Positive Control", "Negative Control" and "Test Inhibitor" conditions.

1. Seed TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells at a density of ~35,000 cells/well in 100 µl of Thaw Medium 1 into a clear-bottom, white 96-well plate. Include wells for a "Positive Control" (effect of the presence of agonist) and "Negative Control" (basal SBE reporter activity in the absence of agonist). Leave a few cell-free wells as "Luminescence Background Control".

   Note: The cells must be treated with an agonist to measure the effect of an inhibitor.

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

3. 24 hours later, prepare a serial dilution of the test inhibitor (for example, TGFβ receptor I inhibitor SB525334) (90 µl/well).

   3.1 For a small molecule inhibitor soluble in media, prepare a serial dilution in Thaw Medium 1B. For controls use Assay Medium 1B (Diluent Solution).

   OR

   3.2 For a small molecule inhibitor soluble in DMSO, prepare a stock solution in 100% DMSO at a concentration 1,000x higher than the highest desired final concentration, then dilute 1,000-fold with Thaw Medium 1B to prepare the highest concentration of the serial dilution. The concentration of DMSO is now 0.1% DMSO.

   Use Assay Medium 1B + 0.1% DMSO to prepare the serial dilution and keep the concentration of DMSO constant.

   For controls use Assay Medium 1B + 0.1% DMSO (Diluent Solution).

   Note: The concentration of DMSO should not exceed 0.1% in the final reaction.

4. Carefully remove the medium from all wells.

5. Add 90 µl of each inhibitor dilution to the wells labeled as "Test Inhibitor" (the inhibitor is now at 1.1x).

6. Add 90 µl of Diluent Solution to the wells labeled as "Positive Control" and "Negative Control".

7. Incubate the cells at 37°C in a 5% CO_2 incubator for ~4-5 hours.

8. Prepare the desired agonist at a concentration 10-fold higher than the desired final concentration using Assay Medium 1B (for example, prepare human TGFβ1 at 200 ng/ml for a final concentration of 20 ng/ml).

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9. Add 10 µl of agonist dilution to the wells labeled as “Test Inhibitor” and “Positive Control”.

10. Add 10 µl of Assay Medium 1B to the wells labeled as “Negative Control”.

11. Add 100 µl of Assay Medium 1B to the “Luminescence Background Control”.

12. Incubate the cells at 37°C in 5% CO_2 overnight (~18 hours).

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

14. Measure luminescence using a luminometer.

15. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of SBE Luciferase Reporter Activity is the background-subtracted luminescence of the inhibitor-treated wells divided by the average background-subtracted luminescence of the untreated wells (negative control without inhibitor), multiplied by 100.

$$Percent\ luminescence = \left(\frac{Luminescence\ of\ Inhibitor\ Treated\ Wells - avg.background}{Avg.Luminescence\ of\ Untreated\ Wells - avg.background}\right) x100$$

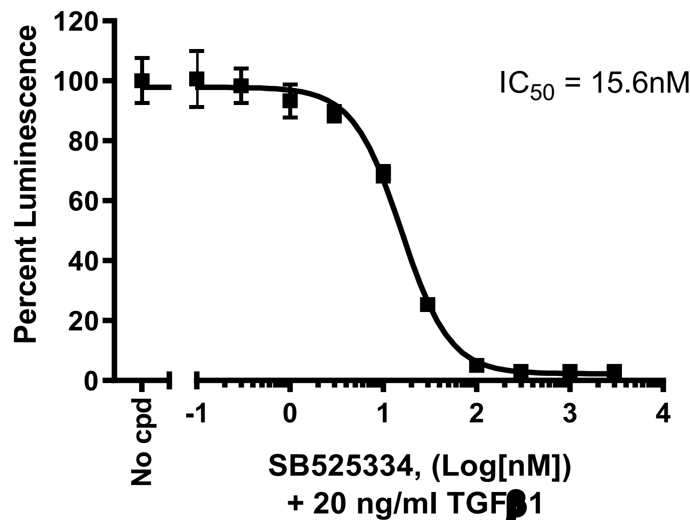

Figure 6: Inhibition of TGFβ1-induced reporter activity by SB525334 in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line.

The cells were incubated with increasing doses of SB525334 prior to stimulation with human TGFβ1. The results are shown as percentage of luminescence in which cells stimulated with TGFβ1 in the absence of SB525334 was set at 100%.

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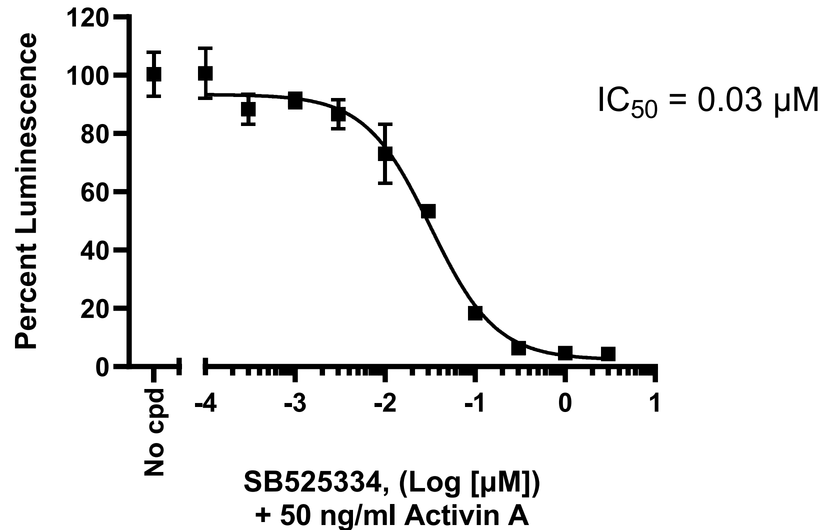

Figure 7: Inhibition of Activin A-induced reporter activity by SB525334 in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line.
The cells were incubated with increasing doses of SB525334 prior to stimulation with human Activin A. The results are shown as percentage of luminescence in which cells stimulated with Activin A in the absence of SB525334 was set at 100%.

C. Inhibition of agonist-induced reporter activity in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by an Anti-ActRII antibody, Bimagrumab

- This experiment measures the effect of anti-ActRII antibodies on agonist induced SBE reporter activation.
- All conditions should be performed in triplicate.
- The assay should include "Luminescence Background Control", "Positive Control", "Negative Control" and "Test Antibody" conditions.

1. Seed TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells at a density of ~35,000 cells/well in 100 µl of Thaw Medium 1 into a clear-bottom, white 96-well plate. Include wells for a "Positive Control" (effect of the presence of agonist) and "Negative Control" (basal SBE reporter activity in the absence of agonist). Leave a few cell-free wells as "Luminescence Background Control".

   Note: The cells must be treated with an agonist to measure the effect of an inhibitor.

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

3. 24 hours later, prepare a serial dilution of the test antibody (90 µl/well). For example, do a 5-fold serial dilution of Bimagrumab.

4. Carefully remove the medium from all wells.

5. Add 90 µl of each antibody dilution to the wells labeled as "Test Antibody" (the antibody is now at 1.1x).

6. Add 90 µl of Assay Medium 1B to the wells labeled as "Positive Control" and "Negative Control".

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7. Incubate the cells at 37°C in a 5% CO_2 incubator for ~4-5 hours.

8. Prepare the desired agonist at a concentration 10-fold higher than the desired final concentration using Assay Medium 1B. For example, prepare Activin A at 300 ng/ml for a final concentration of 30 ng/ml.

9. Add 10 µl of agonist dilution to the wells labeled as “Test Antibody” and “Positive Control”.

10. Add 10 µl of Assay Medium 1B to the wells labeled as “Negative Control”.

11. Add 100 µl of Assay Medium 1B to the “Luminescence Background Control”.

12. Incubate the cells at 37°C in 5% CO_2 overnight (~18 hours).

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

14. Measure luminescence using a luminometer.

15. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of SBE Luciferase Reporter Activity is the background-subtracted luminescence of the antibody inhibitor -treated wells divided by the average background-subtracted luminescence of the untreated wells (negative control without antibody inhibitor), multiplied by 100.

$$Percent\ luminescence = \left(\frac{Luminescence\ of\ \ Inhibitor\ Treated\ Wells - avg.background}{Avg.Luminescence\ of\ Untreated\ Wells - avg.background}\right) x100$$

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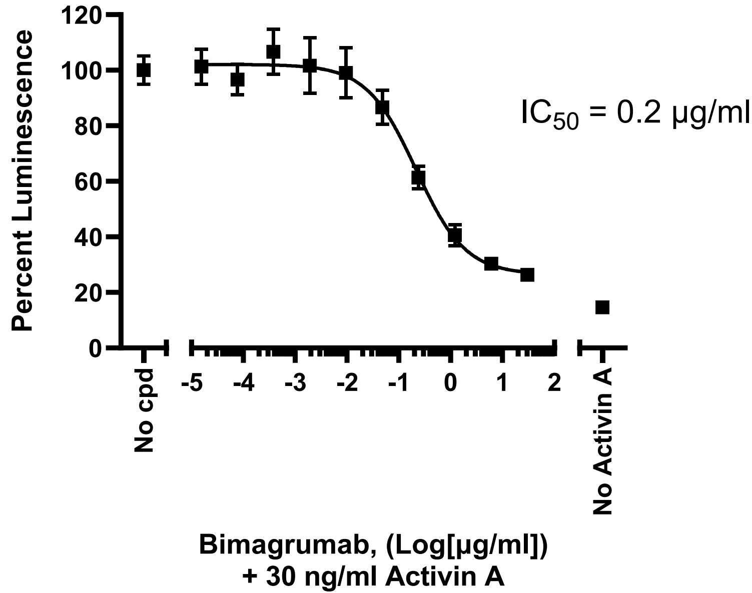

Figure 8: Inhibition of Activin A-induced reporter activity by Bimagrumab in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line.
The cells were incubated with increasing doses of Bimagrumab prior to stimulation with Activin A. The results are shown as percentage of luminescence in which cells stimulated with Activin A in the absence of Bimagrumab was set at 100%.

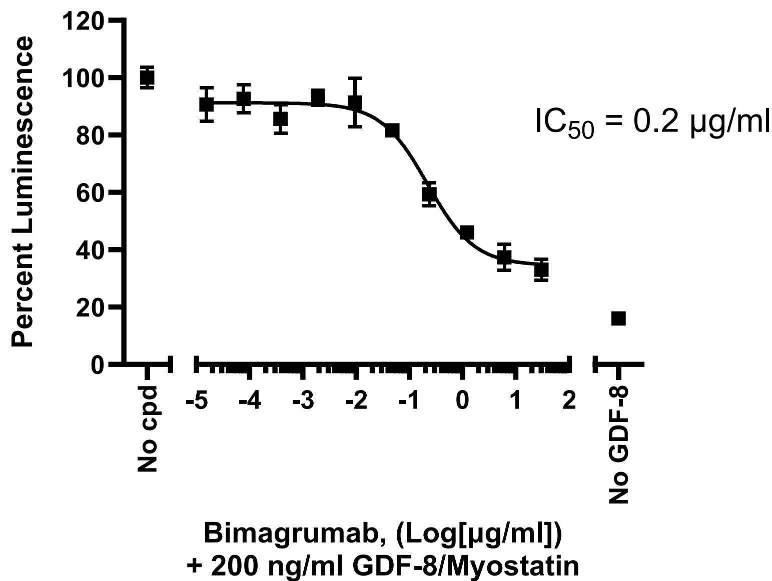

Figure 9: Inhibition of Myostatin-induced reporter activity by Bimagrumab in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line.
The cells were incubated with increasing doses of Bimagrumab prior to stimulation with GDF-8/Myostatin. The results are shown as percentage of luminescence in which cells stimulated with GDF-8/Myostatin in the absence of Bimagrumab was set at 100%.

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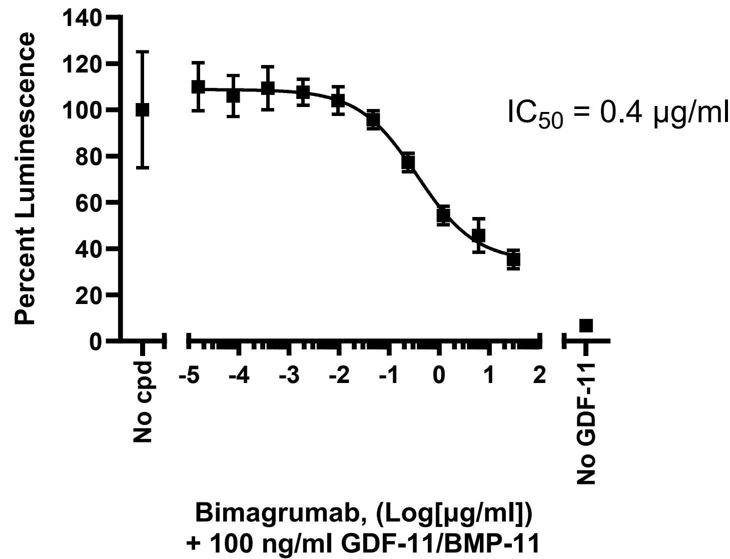

Figure 10: Inhibition of BMP-11-induced reporter activity by Bimagrumab in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line.
The cells were incubated with increasing doses of Bimagrumab prior to stimulation with GDF-11/BMP-11. The results are shown as percentage of luminescence in which cells stimulated with GDF-11/BMP-11 in the absence of Bimagrumab was set at 100%.

D. Inhibition of Activin A-induced reporter activity in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by an Activin Receptor Type IIA-Fc Fusion Protein (Activin Blocker)

- This experiment measures the effect of a recombinant ActRIIA-Fc fusion protein (Activin Blocker) on agonist induced SBE reporter activation.
- All conditions should be performed in triplicate.
- The assay should include “Luminescence Background Control”, “Positive Control”, “Negative Control” and “Test Inhibitor” conditions.

1. Seed TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells at a density of ~35,000 cells/well in 100 µl of Thaw Medium 1 into a clear-bottom, white 96-well plate. Include wells for a “Positive Control” (effect of the presence of agonist) and “Negative Control” (basal SBE reporter activity in the absence of agonist). Leave a few cell-free wells as “Luminescence Background Control”.

   Note: The cells must be treated with Activin A to measure the effect of the Activin Blocker.

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

3. 24 hours later, carefully remove the medium from all wells and replace it with 80 µl of fresh Assay Medium 1B.

4. Incubate cells at 37°C in a 5% CO_2 incubator for ~4 hours.

5. Prepare a serial dilution of the test inhibitor (for example, Activin Blocker) in Assay Medium 1B at concentrations 10-fold higher than the desired final concentrations (10 µl/well).

6. Prepare a dilution of Activin A in Assay Medium 1B at a concentration 10-fold higher than the desired final concentration (final [Activin A] on cells = 30 ng/ml) (10 µl/well).

7. In a separate 96-well plate (Inhibitor + Activin A plate), with no cells, prepare the following:

    a. Add 10 µl/well of the diluted test inhibitor to the "Test Inhibitor" wells.

    b. Add 10 µl/well of Assay Medium 1B to the "Positive Control" wells.

    c. Add 20 µl/well of Assay Medium 1B to the "Negative Control" wells.

    d. Add 10 µl/well of diluted Activin A to the "Test Inhibitor" and "Positive Control" wells.

8. Pre-incubate the Inhibitor + Activin A plate at 37°C in a 5% CO_2 incubator for ~1 hour.

9. Transfer 20 µl of each well of the Inhibitor + Activin A plate to the corresponding wells of the plate containing the cells. The final volume in each well will be 100 µl.

10. Add 100 µl of Assay Medium 1B to the "Luminescence Background Control" wells.

11. Incubate the cells at 37°C in 5% CO_2 overnight (~18 hours).

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

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of SBE Luciferase Reporter Activity is the background-subtracted luminescence of the inhibitor-treated wells divided by the average background-subtracted luminescence of the untreated wells (negative control without inhibitor), multiplied by 100.

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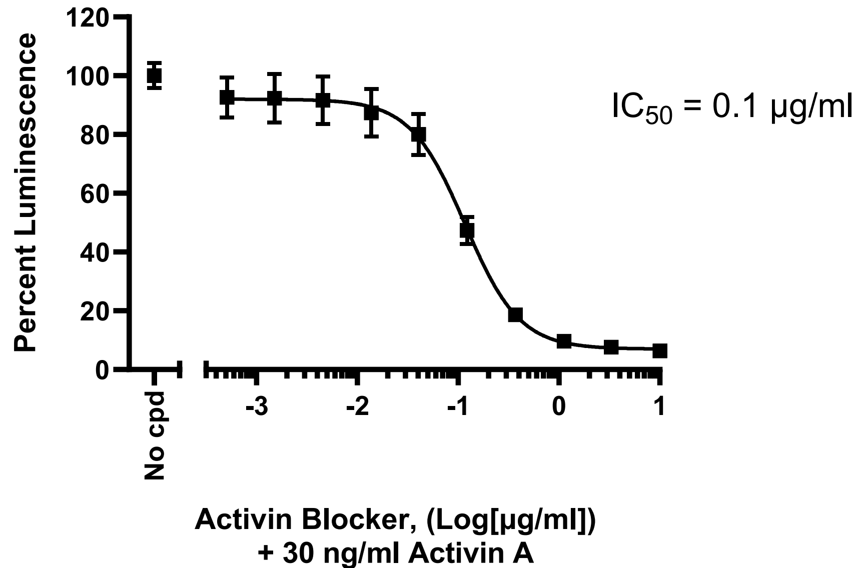

Figure 11: Inhibition of Activin A-induced reporter activity by Activin Blocker in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line.
The cells were incubated with increasing doses of Activin Blocker (#102121) prior to stimulation with Activin A. The results are shown as percentage of luminescence in which cells stimulated with Activin A in the absence of Activin Blocker was set at 100%.

E. Inhibition of TGFβ-induced reporter activity in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line by a human IgG4 Anti-TGFβ Antibody

- All conditions should be performed in triplicate.
- The assay should include “Luminescence Background Control”, “Positive Control”, “Negative Control” and “Test Antibody” conditions.

1. Seed TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 cells at a density of ~35,000 cells/well in 100 µl of Thaw Medium 1 into a clear-bottom, white 96-well plate. Include wells for a “Positive Control” (effect of the presence of agonist) and “Negative Control” (basal SBE reporter activity in the absence of agonist). Leave a few cell-free wells as “Luminescence Background Control”.

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

3. 24 hours later, carefully remove the medium from all wells and replace it with 80 µl of fresh Assay Medium 1B.

4. Incubate cells at 37°C in a 5% CO_2 incubator for ~4 hours.

5. Prepare a serial dilution of the test antibody in Assay Medium 1B at concentrations 10-fold higher than the desired final concentrations (10 µl/well).

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6. Prepare a dilution of TGFβ in Assay Medium 1B at a concentration 10-fold higher than the desired final concentration (final [TGFβ] on cells = 20 ng/ml) (10 µl/well).

7. In a separate 96-well plate (Antibody + TGFβ plate), with no cells, prepare the following:

    a. Add 10 µl/well of the diluted test antibody to the "Test Antibody" wells.

    b. Add 10 µl/well of Assay Medium 1B to the "Positive Control" wells.

    c. Add 20 µl/well of Assay Medium 1B to the "Negative Control" wells.

    d. Add 10 µl/well of diluted TGFβ to the "Test Antibody" and "Positive Control" wells.

8. Pre-incubate the Antibody + TGFβ plate at 37°C in a 5% CO_2 incubator for ~1 hour.

9. Transfer 20 µl of each well of the Antibody + TGFβ plate to the corresponding wells of the plate containing the cells. The final volume in each well will be 100 µl.

10. Add 100 µl of Assay Medium 1B to the "Luminescence Background Control" wells.

11. Incubate the cells at 37°C in 5% CO_2 overnight (~18 hours).

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

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence from the luminescence reading of all other wells. The percent luminescence of SBE Luciferase Reporter Activity is the background-subtracted luminescence of the inhibitor-treated wells divided by the average background-subtracted luminescence of the untreated wells (negative control without inhibitor), multiplied by 100.

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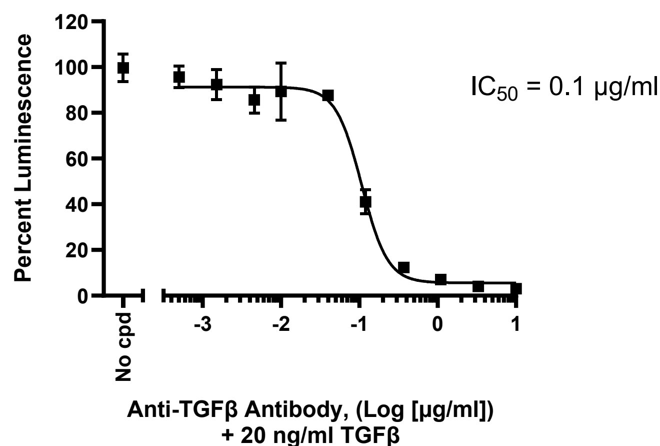

Figure 12: Inhibition of TGFβ-induced reporter activity by Anti-TGFβ Antibody in the TGFβ/Activin A/Myostatin-Responsive Luciferase Reporter HEK293 Cell Line.
The cells were incubated with increasing doses of Anti-TGFβ Antibody (#102330) prior to stimulation with TGFβ. The results are shown as percentage of luminescence in which cells stimulated with TGFβ in the absence of Anti-TGFβ Antibody was set at 100%.

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

References

Moustakas A, *et al.,* 2001 *J. Cell Science.* 114: 4359-69.

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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*
TGFβ1, Latent	90901	5 µg/25 µg/1 mg
TGFβR1, Fc fusion, Avi-Tag Recombinant	100644	20 µg
TGFβR2, GST-Tag Recombinant	40707	50 µg
PAI-1 (Luc) Reporter Mv1-Lu Cell Line	60544	2 vials
SBE Luciferase Reporter Lentivirus (TGFβ/SMAD Pathway)	79806	500 µl x 2
TGFBR2 CRISPR/Cas9 Lentivirus (Non-Integrating)	78536	500 µl x 2
TGFBR2 CRISPR/Cas9 Lentivirus (Integrating)	78535	500 µl x 2

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