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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Activin A: Activin RIIB [Biotinylated] Inhibitor Screening Colorimetric Assay Kit is a colorimetric-based assay designed to measure the binding between human Activin A and Activin RIIB (receptor IIB), also known as ACVR2B, for screening and profiling applications. This kit comes with enough purified Activin A (amino acids 311-426) and biotinylated Activin Receptor IIB (amino acids 19-137), streptavidin-HRP, assay buffer, and detection reagent for 100 enzyme reactions.

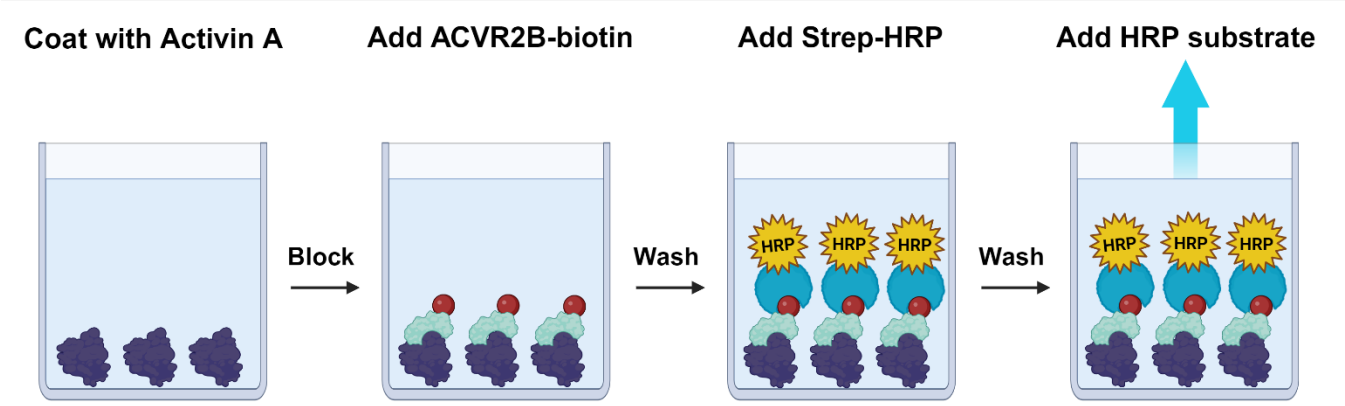

Figure 1. Activin A: Activin RIIB[Biotinylated] Inhibitor Screening Colorimetric Assay Kit schematic.
A 96-well plate is coated with Activin A protein. After blocking, biotinylated Activin RIIB (ACVR2B) is added in an optimized assay buffer. Next, unbound biotinylated Activin RIIB is removed by washing, and the plate is incubated with streptavidin-HRP. After a final wash, colorimetric HRP substrate is added to produce color, which is proportional to the binding of ACVR2B with Activin A.

Background

Activin A is a member of the TGFβ (transforming growth factor beta) family of proteins involved in embryonic development, hematopoiesis, cell proliferation, and cell differentiation. It is the ligand to the activin A receptor type I or type II, which are transmembrane receptors with serine/threonine kinase activity. Upon binding of activin, the kinase activity of the receptor is activated, SMAD2 (mothers against decapentaplegic homolog 2) and SMAD3 are phosphorylated, form a complex with SMAD4 and translocate to the nucleus, regulating gene expression. Activin A can be found in macrophages, dendritic cells, and neutrophils, playing a role in cell maturation and activation. It is involved in inflammation and autoimmune disorders, such as SLE (systemic lupus erythematosus), RA (rheumatoid arthritis), and atopic dermatitis. It is also involved in bone formation. The use of fusion protein blockers of the activin signaling pathways, that serve as sinks for activin A and other TGFβ members, is being explored for the treatment of pulmonary hypertension, chemotherapy-induced anemia, and osteoporosis.

Applications

Study and screen compounds that inhibit the binding of Activin A to Activin RIIB for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
102016	Activin A (Active), His-Tag*	5 µg	-80°C
	Activin RIIB, His-tag, Avi-tag, Biotin Labeled*	1 µg	-80°C
82620	5x PP-02 Buffer	4 ml	-80°C
79743	Blocking Buffer 3	50 ml	-80°C
79742	Streptavidin HRP	10 µl	-80°C
79651	Colorimetric HRP Substrate	10 ml	+4°C
79964	96-well transparent plate	1	Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*
- 2 M sulfuric acid (aqueous)
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

**Alternately, a spectrophotometer reading at 650 nm may be used, but sensitivity of the assay will be greatly reduced.*

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This assay kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

- We recommend using Activin Blocker (#102121) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Coat 96-well plate

Coat the plate one day prior to running your samples.

1. Thaw Activin A on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute Activin A protein to 1 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted Activin A to every well, except "Blank" wells.
4. Add 100 µl of Blocking Buffer 3 to the "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of PBST Buffer per well.
7. Tap the plate onto a clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 3 to every well.
9. Incubate at Room Temperature (RT) for at least 90 minutes.
10. Wash the plate three times using 200 µl of PBST Buffer per well.
11. Tap the plate onto a clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Prepare 1x Assay Buffer by diluting 5x PP-02 Assay Buffer 5-fold with distilled water.
2. Add 20 µl of 1x Assay Buffer to every well.
3. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

   For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

OR

3.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Assay Buffer (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of Test Inhibitor to each well labeled as "Test Inhibitor".
5. Add 5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
6. Thaw Activin RIIB on ice. Briefly spin the tube containing the protein to recover its full content.
7. Dilute Activin RIIB to 0.4 ng/µl with 1x Assay Buffer (25 µl/well).
8. Add 25 µl of diluted Activin RIIB to all wells.
9. Incubate at RT for 1 hour.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted Activin RIIB (0.4 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

10. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto a clean paper towel.

Step 3: Detection

1. Dilute 1000-fold the Streptavidin-HRP with Blocking Buffer 3 (50 µl/well).
2. Add 50 µl of diluted Streptavidin-HRP to every well.

3. Incubate for 1 hour at RT.

4. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto a clean paper towel.

5. Add 100 µl of the colorimetric HRP substrate to each well.

6. Incubate the plate at RT until a blue color has developed in the "Positive Control" wells.

   Note: If blue color is visible and strong, the plate can be read immediately at 650 nm using a UV/Vis spectrophotometer microplate reader. If color is too faint, proceed to next step.

7. Add 100 µl of 2 M sulfuric acid to each well.

8. Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader.

   Note: The "Blank" absorbance value should be ~0.05 at 450 nm. Alternatively, the plate may be read at 650 nm without adding 2 M sulfuric acid, but the Signal-to-Background ratio will be decreased.

Example Results

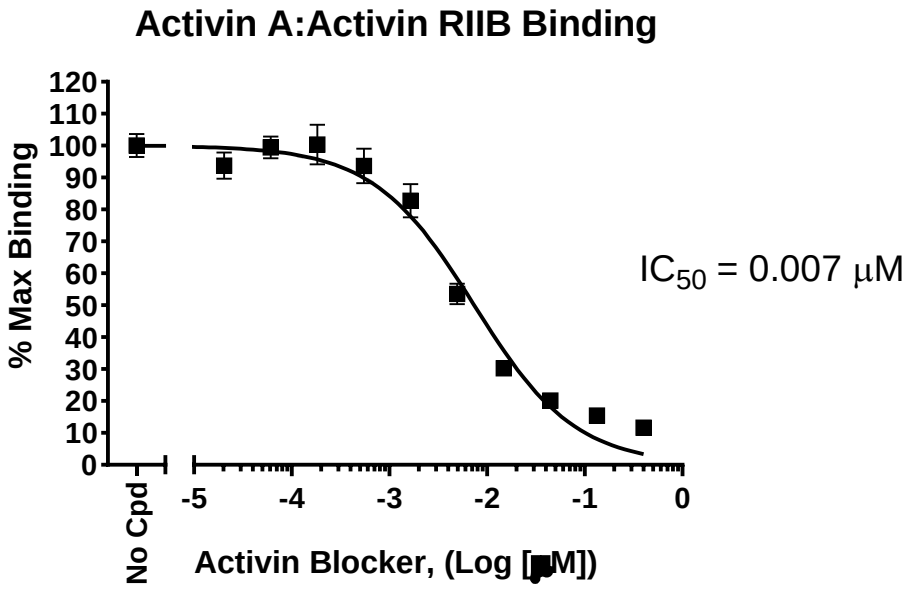

Figure 2: Inhibition of Activin A-Activin RIIB binding by Activin Blocker.
Activin RIIB was incubated with increasing concentrations of Activin Blocker (#102121) in an Activin A coated plate. Results are expressed as a percentage of binding activity in which the condition without Activin Blocker is set to 100%.

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

References

Lodberg A., 2021 *Cytokine & Growth Factor Reviews* 60:1-17.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Activin Blocker	102121	25 µg/ 100 µg
Activin A: Activin RIIB[Biotinylated] Inhibitor Screening Colorimetric Assay Kit	82531	96 reactions
TGFβ/ Activin A-Responsive Luciferase Reporter HEK293 Cell Line	60653	2 vials
ALK2 (ACVR1), GST-tag, Recombinant	40019	10 µg
ALK2 (ACVR1) Assay Kit	79605	96 reactions
ALK1 (ACVRL1) Assay Kit	79549	96 reactions

Version 101724