# Activin A: Activin RIIB[Biotinylated] Inhibitor Screening Assay Kit

#82531
96 reactions

**Description**

The Activin A: Activin RIIB [Biotinylated] Inhibitor Screening Assay Kit is an ELISA-based assay designed to measure the binding between Activin A and Activin RIIB (receptor IIB), also known as ACVR2B, protein 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.

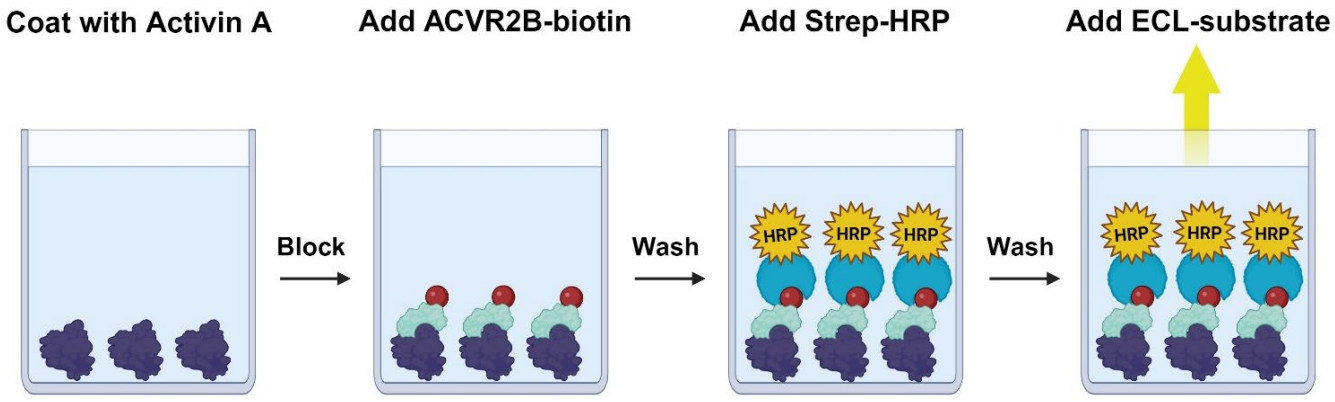


*Figure 1. Activin A: Activin RIIB[Biotinylated] Inhibitor Screening Assay Kit schematic.*
A 96-well plate is coated with Activin A protein. After coating and blocking, biotinylated Activin RIIB, also known as ACVR2B, is added in an optimized assay buffer. Next, unbound biotinylated Activin RIIB is washed away, and the plate is incubated with streptavidin-HRP. After a final wash, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the efficacy of Activin RIIB binding to 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 |
|---|---|---|---|
| | Activin A | 1 µg | -80°C |
| | Activin RIIB, His-Tag, Avi-Tag, Biotin-Labeled* | <1 µg | -80°C |
| | 5x PP-02 Buffer | 4 ml | -80°C |
| 79743 | Blocking Buffer 3 | 50 ml | -80°C |
| 79742 | Streptavidin HRP | 10 µl | -80°C |
| 79670 | ELISA ECL Substrate A (translucent bottle) | 6 ml | Room Temp |
| | ELISA ECL Substrate B (brown bottle) | 6 ml | Room Temp |
| 79837 | 96-well module 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)
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

**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**

The Activin A:Activin RIIB Binding 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 0.2 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 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 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 0.12 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 (0.12 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 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 clean paper towel.
5. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
6. Add 100 µl of mix to every well.
7. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

8. The "Blank" value should be subtracted from all other values.

**Reading Chemiluminescence**

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

**Example Results**

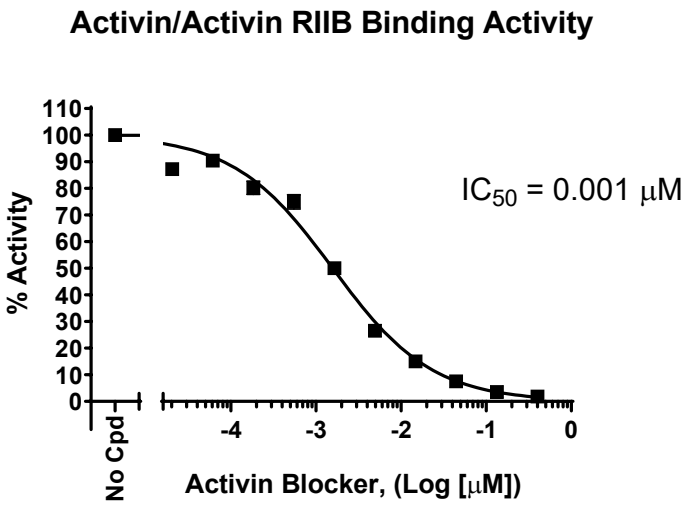


*Figure 2: Inhibition of Activin A-Activin RIIB binding by Activin Blocker.*
Activin RIIB was incubated with increasing concentrations of Activin Blocker (#102121) in an Activin A coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding 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 |
| 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 |

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