BAFF:BAFFR [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit

#82536
96 reactions

Description

The BAFF:BAFFR [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is designed for screening and profiling molecules that block the binding between BAFF (B-cell Activating Factor) and BAFFR (BAFF receptor). This kit comes in a convenient 96-well format, with enough recombinant human biotin-labeled BAFFR (amino acids 23-71), BAFF (amino acids 134-285), streptavidin-HRP, and assay buffer for 100 reactions.

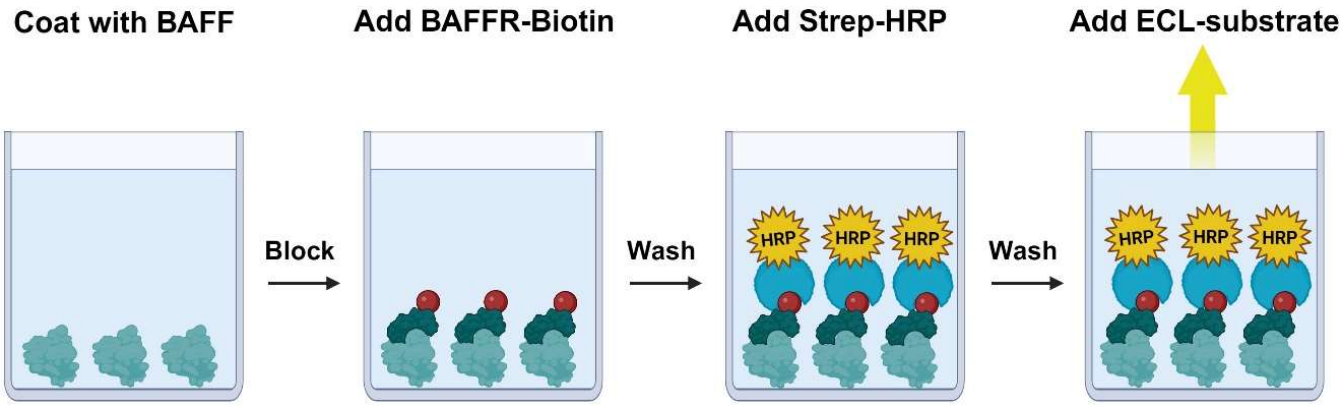

Figure 1: Illustration of the mechanism of BAFF:BAFFR [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.

A 96-well plate is coated with BAFF protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. After incubation with Biotin-BAFFR, the plate is washed and Streptavidin-HRP is added. The ELISA ECL substrate is added, and the resulting signal can be measured using a chemiluminescence microplate reader. The chemiluminescence signal is proportional to the binding of BAFF to BAFFR.

Background

The BAFF (TNF family ligand B-cell Activating Factor), also known as BLyS, TALL-1 or CD257, is encoded by the TNFSF13B gene and is a Type II membrane-bound protein, which can be released as a soluble ligand upon proteolytic processing. This cytokine is a ligand for the receptors Transmembrane Activator and CAML Interactor (TACI or TNFRSF13B), BAFF Receptor (BAFFR, or TNFRSF13C) and B-cell Maturation Antigen (BCMA, TNFRSF17). These interactions promote cell survival and play a crucial role in B cell development. In particular, BAFFR signaling is an important player in the later stages of B cell differentiation, including the survival of long-lived bone marrow plasma cells and likely for the survival of plasma blasts. BAFF signaling is also required for CCL (chronic lymphocytic leukemia) cell survival. Binding of BAFF to BAFFR results in the degradation of TRAF3 (TNF receptor associated factor 3), which stops the inhibition of SYK and results in activation of pathways necessary for B cells. It also activates the non-canonical slow NF-κB2-dependent pathway, which relies on the activation of NIK (NF-κB-inducing kinase). Targeting BAFF-BAFFR will open new therapeutic avenues in diseases related to these proteins.

Application(s)

Screen small molecule inhibitors or antibodies that block BAFF binding to BAFFR.

BPS Bioscience
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Supplied Materials

Catalog #	Name	Amount	Storage
100194	BAFF, His-Avi-Tag*	20 µg	-80°C
100287	BAFF-R, Fc Fusion (IgG1), Avi-Tag, Biotin-Labeled*	5 µg	-80°C
	5x PP-02 Buffer	4 ml	-20°C
	Blocking Buffer 7	40 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrates A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrates B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

**The initial concentration of both BAFF and BAFFR is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- 1x PBS buffer (Phosphate Buffer Saline, pH 7.4)
- PBS-T (1x PBS buffer with 0.05% Tween-20)
- 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 DMSO concentration in the final reaction should be ≤1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Non-Coated Control”, “Blank”, “Positive Control” and “Test Compound” wells.
- We recommend pre-incubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

- We recommend using Anti-BAFF Neutralizing Antibody (#102205) as internal control. If not running a dose response curve, we recommend running the antibody at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1 - Plate coating

1. Thaw **BAFF** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **BAFF** protein to 4 ng/µl in PBS (50 µl/well).
3. Add 50 µl of diluted **BAFF** protein solution to each well.
4. Add 50 µl of PBS to the "Non-Coated Control" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times with 200 µl of PBS-T Buffer per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Add 100 µl of Blocking Buffer 7 to every well.
9. Incubate for 90 minutes at Room Temperature (RT) with gentle agitation.
10. Tap the plate onto clean paper towel to remove the liquid.
11. Start your testing immediately.

Step 2: Reaction

1. Prepare **1x PP-02 Buffer** by diluting 5-fold the **5x PP-02 Buffer** with distilled water.
2. Prepare the Test Compound (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.

   2.1 If the Test Compound is water-soluble, prepare serial dilutions in 1x PP-02 Buffer at concentrations 10- fold higher than the desired final concentrations.

   OR

   2.2 If the Test Compound is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the compound 10-fold in 1x PP-02 Buffer to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

Using 1x PP-02 Buffer containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Compound at 10-fold the desired final concentrations.

For positive and negative controls, prepare 10% DMSO in 1x PP-02 Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

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

3. Add 20 µl of 1x PP-02 Buffer to "Non-Coated Control", "Positive Control" and "Test Compound" wells.
4. Add 45 µl of 1x PP-02 Buffer to "Blank" wells.
5. Add 5 µl of the test compound to each well designated “Test Compound”.
6. Add 5 µl of Diluent Solution to "Non-Coated Control", "Positive Control" and "Blank" wells.
7. Incubate the plate for 30 minutes at RT with gentle agitation.
8. Thaw the **BAFFR-Biotin** on ice. Briefly spin the tube to recover the full content.
9. Dilute **BAFFR-Biotin** to 2 ng/µl with 1x PP-02 Buffer (25 µl/well).
10. Add 25 µl of diluted **BAFFR-Biotin** to the “Non-Coated Control, “Positive Control,” and “Test Compound” wells.
11. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	**Non-Coated Control**	**Positive Control**	**Test Compound**
PP-02 Buffer	45 µl	20 µl	20 µl	20 µl
Test Compound	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
Pre-incubate 30 minutes at RT				
Diluted BAFFR-Biotin (2 ng/µl)	-	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 200 µl of PBS-T Buffer per well.
13. Tap the plate onto clean paper towel to remove the liquid.
14. Block by adding 100 µl of Blocking Buffer 7 to every well and incubate for 10 minutes at RT.
15. Tap the plate onto clean paper towel to remove the liquid.

Step 3: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 7 (50 µl/well).
2. Add 50 µl of diluted **Streptavidin-HRP** to each well.
3. Incubate the plate for 1 hour at RT with gentle agitation.
4. Wash the plate three times with 200 µl of PBS-T Buffer per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
7. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.
8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.
9. The "Blank" value should be subtracted from all readings.

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

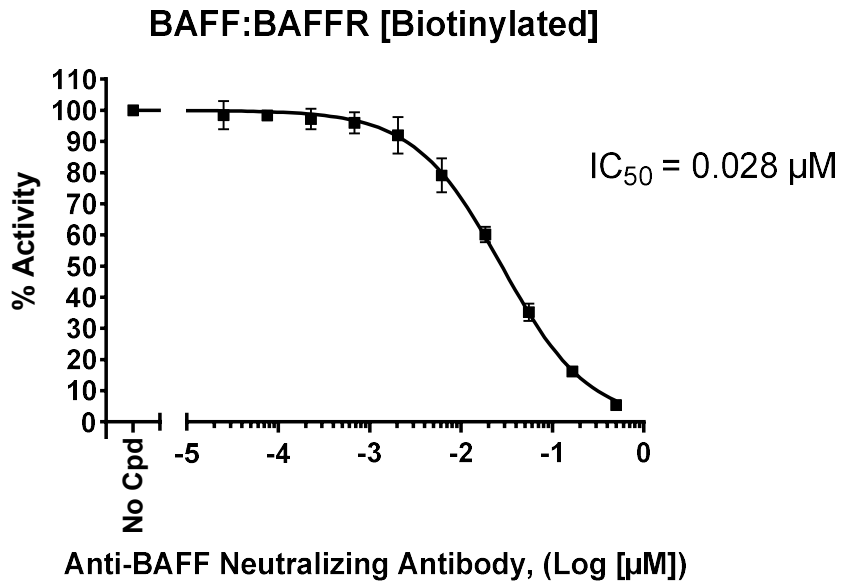

Figure 2. Inhibition of binding of BAFF to BAFFR by Anti-BAFF Neutralizing Antibody.
BAFF:BAFFR binding was evaluated in the presence of increasing concentrations of Anti-BAFF Neutralizing Antibody (#102205). Results are expressed as percent activity, in which binding activity in the absence of neutralizing antibody is set to 100%.

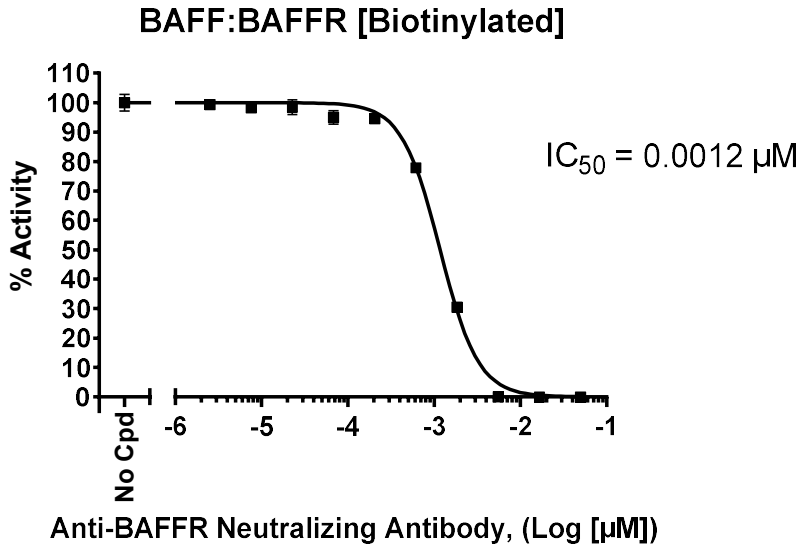

Figure 3. Inhibition of binding of BAFF to BAFFR by Anti-BAFFR Neutralizing Antibody.
BAFF:BAFFR binding was evaluated in the presence of increasing concentrations of Anti-BAFFR Neutralizing Antibody (Abeomics #12-9105). Results are expressed as percent activity, in which binding activity in the absence of neutralizing antibody is set to 100%.

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

Troubleshooting Guide

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

References

Thompson J. S., *et al.*, 2000 *J. Exp. Med.* 192(1): 129-136.
Bossen C., *et al.*, 2006 *Semin. Immunol.* 18(5): 263-275.
Scheighoffer E. and Tybulewicz V., 2021 *Curr Opin Immunol.* 71:124-131.

Related Products

Products	*Catalog #*	*Size*
BAFF-R, Fc-fusion (IgG1), Avi-Tag Recombinant	100286	100 µg
BAFF:BCMA[Biotinylated] Inhibitor Screening Assay Kit	79667	96 reactions
BAFF, His-Avi-Tag Recombinant	100194	50 µg
NIK, GST-Tag Recombinant	40090	10 µg
MAP3K14 Kinase Assay Kit	78440	96 reactions

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