TACI: BAFF [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit

#83729
96 reactions

Description

TACI: BAFF [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is an ELISA-based assay designed to measure the binding between BAFF (B-cell Activating Factor) and TACI (Transmembrane activator and CAML interactor) for screening and profiling applications. This kit comes with enough purified TACI (amino acids 23-166) and biotinylated BAFF (amino acids 134-285), streptavidin-HRP, assay buffer, and detection reagent for 100 enzyme reactions.

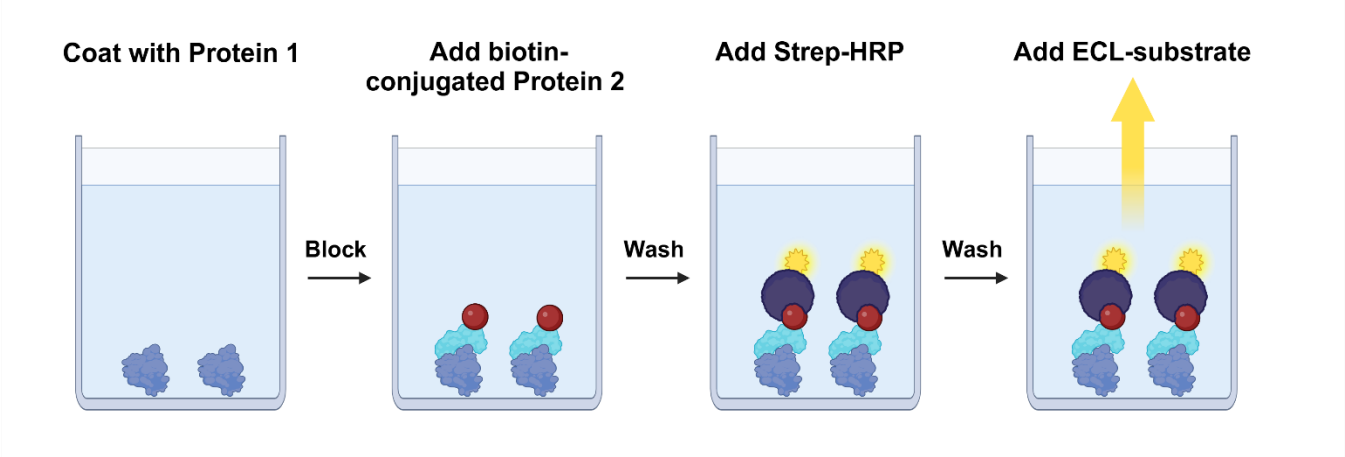

Figure 1. TACI: BAFF [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit schematic.
A 96-well plate is coated with TACI protein. After coating and blocking, biotinylated BAFF is added in an optimized assay buffer. Next, unbound biotinylated BAFF is washed away, and the plate is incubated with 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 BAFF binding to TACI.

Background

TACI (Transmembrane activator and CAML interactor), also known as tumor necrosis factor receptor superfamily member 13B (TNFRSF13B), is a lymphocyte-specific member of the TNF receptor superfamily, which regulates B and T cell function and humoral immunity. This receptor binds to ligands APRIL (A proliferation-inducing ligand) and BAFF, two ligands that also bind to BCMA (B-cell maturation antigen) to induce B cell proliferation. TACI induces antibody responses and plasma cell differentiation, and it counteracts BAFF-driven B cell activation. Upon ligand binding, TACI interacts with CAML (calcium-modulator and cyclophilin ligand) to promote calcineurin-dependent activation of transcription factor NFAT (Nuclear Factor of Activated T cells). Although they do not directly cause disease, mutations in the TNFRSF13B gene predispose patients with CVID (common variable immunodeficiency) to autoimmune and immune cell proliferation disorders, such as SLE (systemic lupus erythematosus), lupus nephritis (LN) and RA (rheumatoid arthritis). The development of inhibitors targeting TACI:BAFF will open new therapeutic avenues.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
100283-KC5	TACI, Fc-Fusion (IgG1), Avi-Tag*	5 µg	-80°C
100220-KC2	BAFF, His-Avi-Tag, Biotin-Labeled*	2 µg	-80°C
79311-KC40	3x Immuno Buffer 1	40 ml	-20°C
82719-KC50	Blocking Buffer 7	50 ml	-20°C
79742	Streptavidin HRP	10 µl	-80°C
79670-KC6	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79969	White 96-well microplate	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

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

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- 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 Anti-BAFF Neutralizing Antibody (#102205) as internal control. If not running a dose response curve for the control inhibitor, we recommend running it 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 **TACI** protein on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute **TACI** protein to 1 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of **diluted TACI** to every well, except "Blank" wells.
4. Add 50 µl of **Blocking Buffer 7** 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 towels to remove the liquid.
8. Block the wells by adding 100 µl of **Blocking Buffer 7** to every well.
9. Incubate at Room Temperature (RT) for 1 hour.
10. Wash the plate three times using 200 µl of **PBST Buffer** per well.
11. Tap the plate onto clean paper towels to remove the liquid.

Step 2: Binding reaction

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

   3.1 If the Test Inhibitor/Blocker is soluble in water, prepare a solution of the compound that is 5-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/Blocker 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 20-fold with

1x Assay Buffer (at this step the compound concentration is 5-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 5%.

Prepare serial dilutions of the Test Inhibitor at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 5% 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 10 µl of **Test Inhibitor** to each well labeled as "Test Inhibitor".
5. Add 10 µl of **Diluent Solution** to the "Positive Control" and "Blank" wells.
6. Pre-incubate the plate for 1 hour at RT with gentle agitation.
7. Thaw **BAFF-Biotin** on ice. Briefly spin the tube containing the protein to recover its full content.
8. Dilute **BAFF-Biotin** to 0.625 ng/µl with 1x Assay Buffer (20 µl/well).
9. Add 20 µl of **diluted BAFF-Biotin** to all wells.
10. Incubate at RT for 2 hours with gentle agitation.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	10 µl
Diluent Solution	10 µl	10 µl	-
Pre-incubate 1 hour at RT			
Diluted BAFF-Biotin (0.625 ng/µl)	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

11. Wash the plate three times with 200 µl of **PBST Buffer** per well.
12. Tap the plate onto clean paper towels to remove the liquid.
13. Block the wells by adding 100 µl of **Blocking Buffer 7** to every well and incubate at RT for 10 minutes.
14. Tap the plate onto clean paper towels to remove the liquid.

Step 3: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 7 (100 µl/well).
2. Add 100 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 **PBS**T Buffer per well and tap the plate onto clean paper towels.
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 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 controls.

Example Results

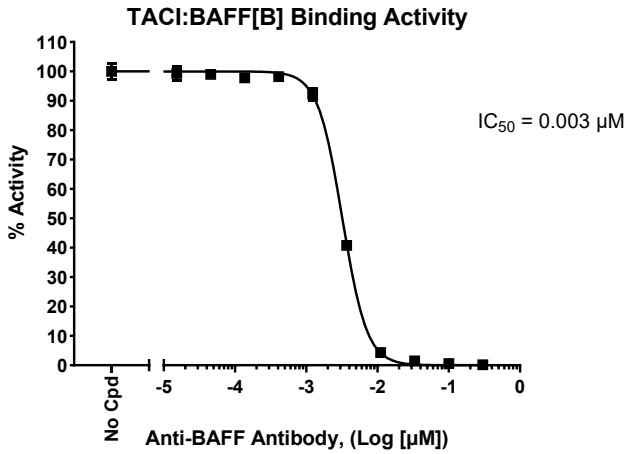

Figure 2: Inhibition of TACI-BAFF binding by Anti-BAFF Neutralizing Antibody.
BAFF was incubated with increasing concentrations of Anti-BAFF Neutralizing Antibody (#102205) in a TACI-coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding activity in which the condition without antibody is set to 100%.

Data shown is representative.

References

Smulski C., *et al.*, 2017 *Cell Reports* 18(9): P2189-2202.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

Related Products

Products	*Catalog #*	*Size*
Anti-BAFF Neutralizing Antibody	102205	25 µg/100 µg/1 mg
BAFF: TACI [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	83631	96 reactions
TACI: APRIL [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	87800	96 reactions
BCMA: APRIL [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	79722	96 reactions
BAFF/APRIL Dual Antagonist	102254	25 µg/ 100 µg/ 1 mg

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