6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data sheet

BAFF:BCMA[Biotinylated] Inhibitor Screening Assay Kit

Catalog #79667
Size: 96 reactions

BACKGROUND: The TNF family ligand B-cell Activating Factor (BAFF, also known as BLyS, TALL-1 or CD257, encoded by the TNFSF13B gene) 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, TNFRSF13B), BAFF Receptor (BAFF-R, TNFRSF13C) and B-cell Maturation Antigen (BCMA, TNFRSF17). These interactions promote cell survival and they play a crucial role in B cell development. In particular, BCMA 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 plasmablasts.

DESCRIPTION: The *BAFF:BCMA[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of BAFF:BCMA signaling. This kit comes in a convenient 96-well format, with biotin-labeled BCMA, purified BAFF, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled BCMA by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, BAFF is coated on a 96-well plate. Next, BCMA is incubated with BAFF on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100194	BAFF, His-Avi-tag HiP™	10 µg	-80 °C	Avoid multiple freeze/thaw cycles!
79467	BCMA, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled HiP™	5 µg	-80 °C	
79311	3x Immuno Buffer 1	50 ml	-20 °C	
	Blocking Buffer	50 ml	+4 °C	
	Streptavidin-HRP	15 µl	-20 °C	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4 °C	
	HRP chemiluminescent substrate B (transparent bottle)	6 ml	+4 °C	
	96-well white microplate	1	+4 °C	

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

APPLICATIONS: This kit is useful for screening for inhibitors of BCMA binding to BAFF.

STABILITY: Up to 6 months from date of receipt, when stored as recommended.

REFERENCES:
Thompson, J. S., *et al*. *J. Exp. Med.* 2000, **192(1)**: 129-136
Bossen, C., *et al. Semin. Immunol.* 2006, **18(5)**: 263-275

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Coating the plate with BAFF:

1) Thaw **BAFF** on ice. Upon first thaw, briefly spin tube containing **BAFF** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **BAFF** in aliquots at -80 °C. Note: **BAFF** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **BAFF** to 2 µg/ml in PBS.
3) Add 50 µl of diluted **BAFF** solution to each well and incubate overnight at 4 °C. Leave a couple of wells empty (uncoated) for use as the “Ligand Control” (see below).
4) Dilute **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with water.
5) Decant to remove supernatant. Wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
6) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 1 hour at room temperature. Remove supernatant as described in step 5.

Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1** + 15 µl distilled water)
2) Add 25 µl of master mixture to each well. Use uncoated wells for the “Ligand Control”.
3) Add 5 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control”, “Ligand Control” and “Blank”, add 5 µl of the same solution without inhibitor (inhibitor buffer). Incubate at room temperature for one hour.

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4) Thaw **BCMA-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **BCMA-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at - 80 °C. Note: ***BCMA-biotin*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer	10 µl	10 µl	10 µl	10 µl
distilled water	15 µl	15 µl	15 µl	15 µl
Test Inhibitor	-	-	-	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
BCMA-Biotin (0.85 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Dilute **BCMA-biotin** to 0.85 ng/µl in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

6) Add 20 µl of **1x Immuno Buffer 1** to the well designated “Blank”.

7) Initiate reaction by adding 20 µl of diluted **BCMA-biotin** (see Step 1-5) to wells labeled “Positive Control”, “Ligand Control” and “Test Inhibitor”. Incubate at room temperature for two hours.

8) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

9) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-8.

Step 2:

1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer**.

2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towel to remove liquid.

4) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

5) Just before use, mix on ice 50 µl **HRP Chemiluminescent Substrate A** and 50 µl **HRP Chemiluminescent Substrate B**, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.

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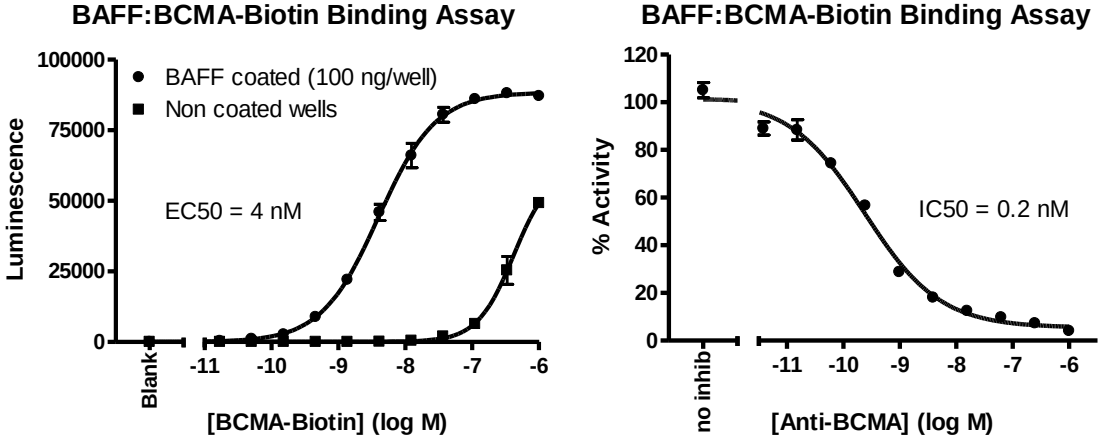
6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

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 of assay results:



BAFF:BCMA binding activity, measured using the using the *BAFF:BCMA[Biotinylated] Inhibitor Screening Assay Kit*, BPS Bioscience #79667 (left). Inhibition of BAFF:BCMA binding using the BCMA Neutralizing Antibody, BPS Bioscience #100173 and the *BAFF:BCMA[Biotinylated] Inhibitor Screening Assay Kit* (right). Luminescence was measured using a Bio-Tek fluorescent microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

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RELATED PRODUCTS:

Product Name	Catalog#	Size
BAFF, His-Avi-Tag HiP™	100194	50 µg
BAFF	90100-1	10 µg
BAFF	90100-2	100 µg
Human BAFF-R (CD268)	90103-B	50 µg
BCMA, Fc-fusion (IgG1), Avi-Tag, Biotin-Labeled HiP™	79467	50 µg
BCMA, Fc-Fusion, Avi-Tag HiP™	79465	100 µg
Anti-BCMA Antibody (scFv) His-Tag	100173-1	50 µg
BCMA CHO Recombinant Cell Line (High Expression)	79500-H	2 vials
BCMA CHO Recombinant Cell Line (Medium Expression)	79500-M	2 vials
BCMA CHO Recombinant Cell Line (Low Expression)	79500-L	2 vials

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TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	BAFF or BCMA has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh BCMA-biotin, (BPS Bioscience #79467) and fresh BAFF (BPS Bioscience #100194). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in PBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of BCMA-biotin (BPS Bioscience #79467) to create a standard curve