EP118-EN.01

# BCMA[Biotinylated]: BAFF Inhibitor Screening Assay Kit

**Pack Size: 96 tests**

**Catalog Number：** EP-118

**IMPORTANT: Please carefully read this manual before performing your experiment.**

***For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures***

## INTENDED USE

This kit is designed for screening of inhibitors of human BCMA binding to human BAFF.

It is intended for research use only (RUO).

## PRINCIPLE OF THE ASSAY

This inhibitor screening Assay kit is designed to facilitate the identification and characterization of new BCMA pathway inhibitors. The assay takes advantage of our in house-developed binding of biotinylated human BCMA to immobilized human BAFF in a functional chemiluminescence assay. Briefly, we provide you with a human Biotinylated BCMA protein, a human BAFF protein, an anti-BCMA neutralizing antibody (as method verified Std.), and Streptavidin-HRP reagent. Your experiment will include 4 simple steps:

1) Coat the plate with human BAFF.

2) Add your molecule of interest to the tests.

3) Add human BCMA-Biotin to bind the coated human BAFF.

4) Add Streptavidin-HRP followed by TMB or other colorimetric HRP substrate.

Finally, the half maximal inhibitory concentration (IC50) of your compound to BCMA: BAFF binding will be determined by comparing OD readings among different experimental groups.

## MATERIALS PROVIDED

**TABLE 1. MATERIALS PROVIDED (pls modify according to COA)**



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| Catalog | Components | Size (96 tests) | Format | Storage | |
|---|---|---|---|---|---|
| EP118-C01 | High-bind Plate | 1 plate | Solid | 2-8°C | |
| EP118-C02 | Human BAFF | 10 μg | Powder | 2-8°C | -70°C after reconstitution, avoid freeze-thaw cycles |
| EP118-C03 | Biotinylated Human BCMA | 10 μg | Powder | 2-8°C | |
| EP118-C04 | Anti-BCMA Neutralizing Antibody | 20 μg | Powder | 2-8°C | |
| EP118-C05 | Streptavidin-HRP | 5 μg | Powder | 2-8°C, avoid light | |
| EP118-C06 | Coating Buffer | 12 mL | Liquid | 2-8°C | |
| EP118-C07 | 10xWashing Buffer | 50 mL | Liquid | 2-8°C | |
| EP118-C08 | Blocking Buffer | 50 mL | Liquid | 2-8°C | |
| EP118-C09 | Substrate Solution | 12 mL | Liquid | 2-8°C | |
| EP118-C10 | Stop Solution | 7 mL | Liquid | 2-8°C, avoid light | |

## REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

A luminometer or microtiter-plate capable of reading chemiluminescence；

Centrifuge；

37 ºC Incubator；

Single channel or multichannel pipettes with 10 μL, 200 μL and 1000 μL precision；

10 μL, 200 μL and 1000 μL pipette tips；

Test Tubes；

Graduated cylinder；

Deionized or distilled water for dilution；

## STORAGE AND VALIDITY INSTRUCTIONS

Unopened kit should be stored at 2°C -8°C upon receiving. Find the expiration date on the outside packaging and do



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not use reagents past their expiration date.

The kit should be stored as TABLE 1 after the reconstitution of lyophilized materials. The shelf life is 30 days from the date of opening.

*Note:*

a. *Do not use reagents past their expiration date.*

b. *Find the expiration date on the outside packaging.*

## REAGENT PREPARATION

1. Restore all reagents and samples to room temperature (20-25℃) before use.

2. Reconstitute the provided lyophilized materials to stock solutions with sterile deionized water as recommended in Tab.2, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortex. The reconstituted stock solutions should be stored at -70℃. **Avoid freeze-thaw cycles**.

***Note: Streptavidin-HRP stock solution should be protected from light***.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

| Catalog | Components | Amount | Stock Solution Con. | Reconstitution Buffer and Vol. |
|---|---|---|---|---|
| EP118-C02 | Human BAFF | 10 μg | 50 μg/mL | 200 μL, water |
| EP118-C03 | Biotinylated Human BCMA | 10 μg | 50 μg/mL | 200 μL, water |
| EP118-C04 | Anti-BCMA Neutralizing Antibody | 20 μg | 100 μg/mL | 200 μL, water |
| EP118-C05 | Streptavidin-HRP | 5 μg | 50 μg/mL | 100 μL, water |

## RECOMMENDED PROTOCOL

### 1. Working solution preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 10×Washing Buffer with ultrapure water/deionized water to 500 mL.

1.2 Preparation of Dilution Buffer:

10 mL Blocking Buffer (EP118-C08) add 30 mL 1×Washing Buffer.

### 2. Coating



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1)Dilute Human BAFF stock solution (100 μg/mL) to 0.5μg/mL with Coating Buffer to make Human BAFF working solution.

2)Add 100 μL of Human BAFF working solution (0.5μg/mL) to each well and leave a couple of wells uncoated for No-Coating Control, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4℃.

## 3. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the washing step above for three times.

*Note: For best results, the complete removal of the Human BAFF solution is essential. The use of a manifold dispenser or an auto-washer may be necessary.*

## 4. Blocking

Add 300 μL Blocking Buffer to each well, seal the plate with microplate sealing film and incubate at 37℃ for 1.5 hours.

## 5. Washing

Repeat step 3. At the same time, you can start to prepare your samples.

## 6. Add Samples

1)Make series dilution of the samples as appropriate.

2)If you intend to use the provided Anti-BCMA Neutralizing Antibody as a reference (Std.), you may dilute the antibody as recommended in Figure 1.

3)Add 50 μL of sample solution to each well according to our recommendation (Figure 2) or your own plate setup.

4)For No-Coating Control wells, please add 50 μL Dilution Buffer.

## 7.Binding

1) Dilute Biotinylated Human BCMA stock solution (50 μg/mL) to 0.03 μg/mL with Dilution Buffer to make Biotinylated Human BCMA working solution.

2) For No-binding ctrl. wells, please add 50 μL Dilution Buffer

3) For all other wells, please add 50 μL Biotinylated Human BCMA working solution to the wells and mix the samples



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by gently tapping the plate. Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

**Note: The working solution should be prepared immediately before use and should not be stored.**

**FIG.1 PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-BCMA Neutralizing Antibody**

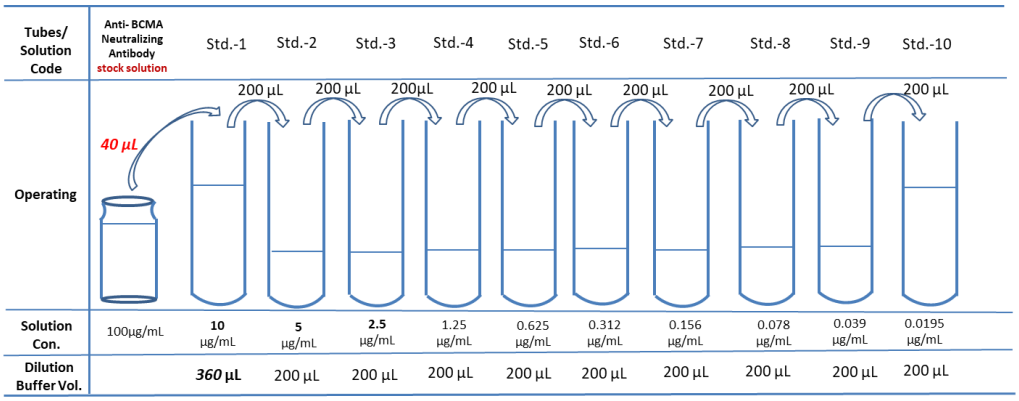
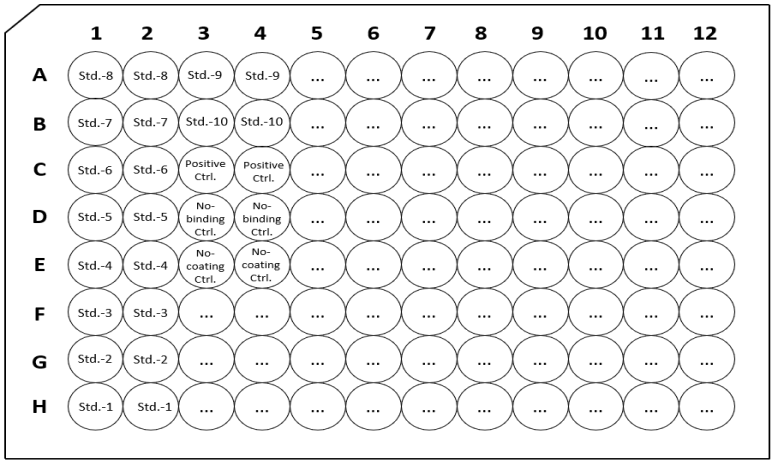



**FIG.2 PLATE LAYOUT**




## 8.Washing

Repeat step 3.

## 9.Add Streptavidin-HRP

1)Dilute Streptavidin-HRP stock solution (50 µg/mL) to 0.1 µg/mL with Dilution Buffer to make Streptavidin-HRP working solution.

2)For all wells, add 100 µL Streptavidin-HRP working solution, seal the plate with microplate sealing film and incubate at 37°C for 1 hour, avoid light.

## 10.Washing



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Repeat step 3.

## 11.Substrate Reaction

Add 100 μL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 minutes. Avoid light.

## 12.Termination

Add 50 μL **Stop Solution** to each well, and gently shake the plate to allow thorough mixing.

***Note:*** *the color in the wells should change from blue to yellow.*

## 13.Data Recording

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

***Note****: Subtracting the value read at* $OD_{450nm}$ *with* $OD_{630nm}$ *can be used to reduce the background noise.*

## SIMPLIFIED PROTOCOL

TABLE. 3 ASSAY PROTOCOL

| Steps Code | Steps | Reagents & Instruments | Reaction Conditions | Samples | No-binding Ctrl. | No-coating Ctrl. | Positive Ctrl. |
|---|---|---|---|---|---|---|---|
| 1 | Working fluid preparation | N/A | N/A | N/A | N/A | N/A | N/A |
| 2 | Coating | Human BAFF Working Solution | 4°C for overnight | 100 μL | 100 μL | — | 100 μL |
| 3 | Washing | 1XWash Buffer | Wash for 3 times | 300 μL | 300 μL | 300 μL | 300 μL |
| 4 | Blocking | Blocking Buffer | 37°C for 1.5 hours | 300 μL | 300 μL | 300 μL | 300 μL |
| 5 | Washing | 1XWash Buffer | Wash for 3 times | 300 μL | 300 μL | 300 μL | 300 μL |
| 6 | Add Samples | Samples | — | 50 μL | — | — | — |
| | | Dilution Buffer | | — | 50 μL | 50 μL | 50 μL |
| 7 | Binding | Biotinylated Human BCMA Working Solution | Mix by gentle tapping, incubate at 37°C for 1 hours | 50 μL | — | 50 μL | 50 μL |
| | | Dilution Buffer | | — | 50 μL | — | — |
| 8 | Washing | 1XWash Buffer | Wash for 3 times | 300 μL | 300 μL | 300 μL | 300 μL |



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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **9** | Streptavidin-HRP | Streptavidin-HRP Working Solution | 37℃ for 1 hours | 100 μL | 100 μL | 100 μL | 100 μL |
| **10** | Washing | 1XWash Buffer | Wash for 3 times | 300 μL | 300 μL | 300 μL | 300 μL |
| **11** | Substrate Reaction | Substrate Solution | 37℃ for 20 minutes | 100 μL | 100 μL | 100 μL | 100 μL |
| **12** | Termination | Stop Solution | Mix by gentle tapping | 50 μL | 50 μL | 50 μL | 50 μL |
| **13** | Data Recording | UV/Vis spectrophotometer | Measure absorbance at 450 nm, with the correction wavelength set at 630 nm | | | | |

***Note for TAB. 3:***

1) *Samples: Your samples of interest.*
2) *No-binding Ctrl.: Reaction without Biotinylated Human BCMA added. The absorbance should be around 0.05(< 0.1) at 450 nm.*
3) *No-coating Ctrl.: Reaction without Human BAFF coated on the wells. The absorbance should be around 0.05(< 0.1) at 450 nm.*
4) *Positive Ctrl.: Determined the max value in 450nm absorbance, when out of inhibitors.*
5) *It is recommended that all samples, controls and standards should be done in duplicates.*

## PRECAUSIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balanced to room temperature (20℃-25℃) before use.
5. This kit should be stored at 2℃-8℃.
6. Please prepare the working solution of each component according to the needs of the experiment. Except for 1x Washing Buffer, all prepared working solution is for one-time use and cannot be stored.

## METHOD VERIFICATION

**INHIBITION OF BCMA [BIOTINYLATED]: BAFF BINDING BY ANTI-BCMA NEUTRALIZING ANTIBODY**



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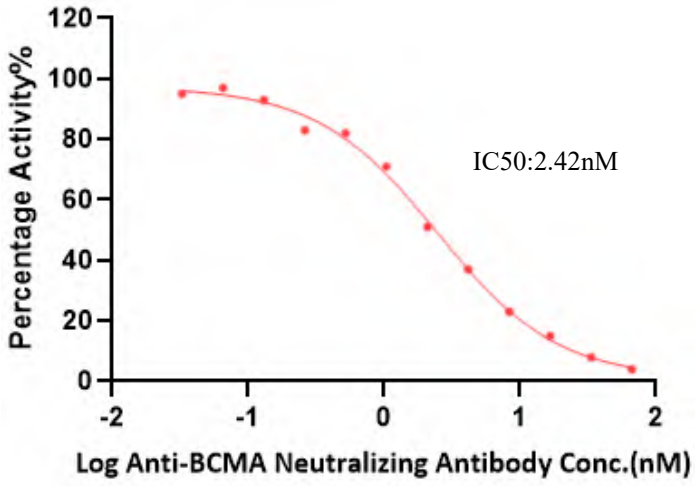
Serial dilutions of Anti-BCMA Neutralizing antibody (Catalog # EP118-C04) (1:1 serial dilution, from 10 μg/mL to 0.0048828125 μg/mL) was added into BAFF: Biotinylated BCMA binding reactions. The assay was performed according to the above-described protocol. Background was subtracted from data points prior to log transformation and curve fitting.

**For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The example data is for reference only.**




| Anti-BCMA Neutralizing Antibody conc.(μg/ml) | Anti-BCMA Neutralizing Antibody conc.(nM) | Mean Abs.(OD450) | Percentage Activity(%) |
|---|---|---|---|
| 0.000 | 0.000 | 2.693 | 100% |
| 0.005 | 0.033 | 2.676 | 99% |
| 0.010 | 0.065 | 2.631 | 98% |
| 0.020 | 0.130 | 2.481 | 92% |
| 0.039 | 0.260 | 2.551 | 95% |
| 0.078 | 0.521 | 2.298 | 85% |
| 0.156 | 1.042 | 2.091 | 78% |
| 0.313 | 2.083 | 1.37 | 51% |
| 0.625 | 4.167 | 1.023 | 38% |
| 1.250 | 8.333 | 0.663 | 25% |
| 2.500 | 16.667 | 0.402 | 15% |
| 5.000 | 33.333 | 0.228 | 8% |
| 10.000 | 66.667 | 0.151 | 6% |
| No Coating | | 0.043 | |
| No Binding | | 0.048 | |



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