Product Manual

BAFF soluble (mouse), detection set

[B Cell Activating Factor, Soluble (mouse) Detection Set; BlyS, Soluble (mouse) Detection Set]

For Research Use Only

APO-54N-013

Version 9 (24 October, 2012)

1. INTENDED USE

The BAFF soluble (mouse), detection set is used for the *in vitro* quantitative determination of soluble mouse BAFF in mouse serum, plasma or cell culture supernatant. This Detection Set is for research use only.

2. INTRODUCTION

BAFF (B cell activation factor of the TNF family, also known as BLyS or TALL1) is a cytokine expressed predominantly by cells of the immune system such as neutrophils, monocytes, macrophages, dendritic cells, follicular dendritic cells, activated T cells and some malignant B cells. BAFF binds three distinct receptors (BAFF-R, TACI and BCMA) expressed predominantly on B cells, although activated T cells also express BAFF-R. BAFF is a master regulator of peripheral B cell survival, and also acts in processes such as immunoglobulin isotype switch and B cell co-stimulation. Besides its major role in B cell biology, BAFF co-stimulates activated T cells. Deregulated expression of this membrane-bound protein, which can readily be released in a soluble form, leads to autoimmune disorders in mice. In humans, elevated levels of soluble BAFF have been detected in the serum or plasma of patients with various autoimmune diseases.

References:

Batten, M., et al. (2004). TNF deficiency fails to protect BAFF transgenic mice against autoimmunity and reveals a predisposition to B cell lymphoma. J. Immunol. 172, 812-822.

Mackay, F., and Ambrose, C. (2003). The TNF family members BAFF and APRIL: the growing complexity. Cytokine Growth Factor Rev. 14, 311-324.

Mackay, F., Sierro, F., Grey, S. T., and Gordon, T. P. (2005). The BAFF/APRIL system: an important player in systemic rheumatic diseases. Curr. Dir. Autoimmun. 8, 243-265.

3. PRINCIPLE OF PROCEDURE

This assay is a sandwich Enzyme-Linked Immuno-Sorbent Assay (ELISA) developed for the direct measurement of mouse BAFF (mBAFF) in serum, plasma and cell culture supernatants. A monoclonal antibody specific for mBAFF is coated onto the wells of microtiter strips. Samples and concentration standards of mBAFF are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, mBAFF is recognized by the addition of a biotinylated monoclonal antibody specific for mBAFF. After removal of excess biotinylated antibody, streptavidin-peroxidase is added. Following a final washing, peroxidase activity is quantified using the substrate 3,3',5,5'-tetramethylbenzidine (TMB). The intensity of the color reaction is measured at 450 nm after acidification and is directly proportional to the concentration of mBAFF in the samples.

4. MATERIALS PROVIDED

- 1 vial (10µg) BAFF, soluble (m) STD
  - (Product No. 80-2345)
  - Store at -20°C (see additional storage requirements in section 7)
- 1 vial (25µg) MAb to BAFF, soluble (m) DET
  - (Product No. 80-2104)
  - Store at -20°C, avoid repeat freeze/thaws
- 2 vials (100µg each) MAb to BAFF, soluble (m) COAT
  - (Product No. 80-2103)
  - Store at -20°C, avoid repeat freeze/thaws

5. SOLUTIONS REQUIRED

- PBS: 137mM NaCl, 2.7mM KCl, 8.1mM Na_2HPO_4, 1.5mM KH_2PO_4, pH 7.2, 0.2µM filtered
- Wash Buffer: 0.1%Tween® 20 in PBS
- Diluent Buffer: 2% BSA in PBS, 0.2µM filtered.
- ELISA Buffer: 0.2% BSA and 0.05% Tween® 20 in PBS
- 1N hydrochloric acid (HCl)
- Tetramethylbenzidine (TMB) substrate (ENZO Product No. ADI-80-0350 or equivalent)
- Streptavidin-peroxidase (SA-HRP) (ENZO Product No. ADI-80-1896 or equivalent)
- HPLC grade water
- Flat-bottom, high binding 96 strip-well microplate (ENZO Product No. ADI-80-1930 or equivalent)

7. GENERAL ELISA PROTOCOL

PREPARATION OF REAGENTS

1. Dilute the desired amount of capture antibody (COAT, 80-2103) (1mg/ml) to 5µg/ml in PBS without carrier protein.
2. Dilute the desired amount of detection antibody (DET, 80-2104) (0.250mg/mL) to 0.250µg/ml in Diluent Buffer
3. Reconstitute the Standard Protein (STD, 80-2345) with 100µl ELISA buffer to obtain a concentration of 100µg/ml. **The reconstituted standard is unstable and must be stored at -20˚C! Avoid freeze/thaw cycles!**
4. A seven point standard curve using 2-fold serial dilutions in ELISA buffer is recommended. Suggested standard points are 20ng/ml, 10ng/ml, 5ng/ml, 2.5ng/ml, 1.25ng/ml, 0.625ng/ml, and 0.3125ng/ml.
5. If measuring serum or cell culture supernatant, dilute samples in ELISA buffer.
6. To reduce interference from rheumatoid factor (RF) in the serum, Ig can be cleared by treating serum dilutions with 0.05 volume of protein G-Sepharose twice for one hour at 4°C before pelleting the beads and collecting supernatants (precleared sera)[1].

PLATE PREPARATION

1. Coat the wells by adding 100µl/well of diluted (5µg/ml) capture antibody (80-2103) to a 96-well ELISA microplate (Costar flat bottom, high binding 96 strip well microplate is suggested). Cover the plate with plastic film and leave overnight at 4°C.
2. Aspirate the coated wells and add 300µl of Wash Buffer using a multichannel pipette or autowasher. Repeat the process for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Buffer by aspirating or by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300µl of Diluent Buffer at room temperature (RT) for 2 hours.

4. At the end of 2 hours, repeat the aspiration/wash as in step 2 for a total of three washes to remove Diluent Buffer.

ASSAY PROCEDURE

1. Add a total of 100µl/well diluted serum, diluted culture supernatant or Standard Protein serial dilutions in ELISA Buffer to the plate.
2. Cover the plate with plastic film and incubate for one hour at RT.
3. Repeat the aspiration/wash as in step 2 of “Plate Preparation” for a total of three washes.
4. Add 100µl/well of the diluted (0.250µg/ml) detection antibody (80-2104).
5. Cover the plate with plastic film and incubate for one hour at RT.
6. Repeat the aspiration/wash as in step 2 of “Plate Preparation” for a total of three washes.
7. Add 100 µl to each well of the diluted HRP Labeled Streptavidin (suggested dilution of ADI-80-1896 is 1:1000).
8. Cover the plate with plastic film and incubate for 30 min at RT.
9. Repeat the aspiration/wash as in step 2 of “Plate Preparation” for a total of four washes.
10. Substrate development occurs by addition of 100µl to each well of tetramethylbenzidine (TMB).
11. Stop the reaction by adding 50µl of 1N HCl. Tap the plate gently to ensure thorough mixing.
12. Measure the OD at 450nm in an ELISA reader.
13. Measure absorbance at 550nm and subtract these values from those obtained at 450nm to correct for optical imperfections in the microplate. If absorbance at 550nm is not possible, measure the absorbance at 450nm only.

Note: When the 550 nm measurement is omitted, absorbance values will be higher.

8. TECHNICAL HINTS AND LIMITATIONS

- Once reagents have been added to the plate, DO NOT let the plate dry at any time during the assay.
- Be sure no sodium azide is present in this assay, as this inhibits HRP enzyme activity.
- It is recommended that all standards and samples be assayed in duplicate.
- Avoid microbial contamination of reagents and equipment. Automated plate washers can become contaminated thereby causing assay variability. Buffers containing a large quantity of protein should be made under sterile conditions and stored at 2-8°C or be prepared fresh daily.
- Vigorous plate washing is essential.
- Avoid exposing reagents to excessive heat or light during storage and incubation.
- Wear gloves while performing the assay to avoid contact with samples and reagents. Please follow proper disposal procedure.

9. CALCULATION OF RESULTS

- Average the duplicate readings for each standard, control and sample and subtract the average blank value.
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding BAFF concentration (ng/ml) on the horizontal axis (see **TYPICAL DATA**).
- Calculate results using graph paper or curve-fitting statistical software (such as Assay Blaster™, ENZO Product No. ADI-28-0002). The amount of mouse BAFF in each sample is determined by interpolating for the absorbance value (Y axis) using the standard curve.
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate ng/ml of mouse BAFF in the sample.

10. TYPICAL DATA

The following data are obtained using the different concentrations of standard as described in this protocol:

Standard mBAFF (ng/mL)	Optical Density (mean)
0	0.084
0.3125	0.121
0.625	0.149
1.25	0.196
2.5	0.345
5	0.602
10	1.088
20	1.98

Please note that these data are provided for illustration only and should NOT be used to calculate results from another assay.

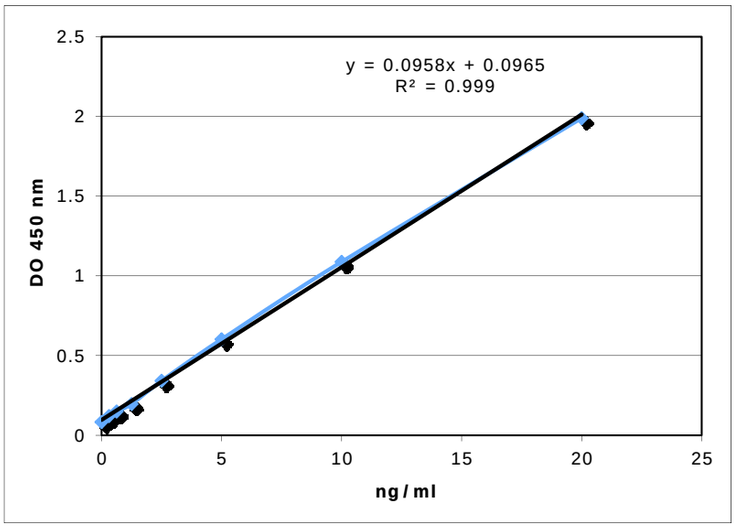

Figure: Standard curve example.

USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences' sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NONINFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending.

www.enzolifesciences.com

Enabling Discovery in Life Science®

Global Headquarters
Enzo Life Sciences Inc.
10 Executive Blvd
Farmingdale, NY 11735
(p) 1-800-942-0430
(f) 1-631-694-7501
(e) info-usa@enzolifesciences.com

Enzo Life Sciences (ELS) AG
Industriestrasse 17, Postfach
CH-4415 Lause / Switzerland
(p) +41/0 61 926 89 89
(f) +41/0 61 926 89 79
(e) info-ch@enzolifesciences.com

Please visit our website at www.enzolifesciences.com for additional contact information.