



**Highest Standards for
Research Kits™**

Manual
BAFF, Soluble (mouse)
ELISA Kit

**[B Cell Activating Factor, Soluble (mouse) ELISA Kit;
BlyS, Soluble (mouse) ELISA Kit]**

For Research Use Only

APO-54N-019-KI01

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1. INTENDED USE

The BAFF, Soluble (mouse) ELISA Kit is to be used for the *in vitro* quantitative determination of soluble mouse BAFF, in mouse serum or cell culture supernatant. This kit 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 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 and cell culture supernatants. A monoclonal antibody specific for mBAFF has been coated onto the wells of the supplied 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, streptavidine-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 mouse BAFF Standard (*lyophilized*) (10 μ g) (STD)
- 1 vial Detection Antibody (20 μ l) (AB)
- 1 vial HRP Labelled Streptavidin (*lyophilized*)(2 μ g) (STREP-HRP)
- 2 bottles Wash Buffer 10X (2 x 30 ml) (Wash Buffer 10X)
- 1 bottle ELISA Buffer 10X (10 ml) (ELISA Buffer)
- 1 bottle Diluent Buffer (15 ml) (Diluent Buffer)
- 1 bottle TMB Substrate Solution (12 ml) (TMB)
- 1 bottle Stop Solution (12 ml) (STOP)
- 1 Plate coated with mBAFF Antibody (6 x 16-well strips)
- 2 Plate Covers (plastic film)
- 2 Silica Gel Minibags

5. MATERIALS NOT PROVIDED

- Calibrated precision pipettes
- Deionized or distilled water
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

6. HANDLING

- Store ELISA kit at 2-8°C.
- Avoid freeze/thaw cycles.
- Plate and reagents should be at room temperature before use.
- Reagents with a volume less than 100 μ l should be centrifuged.
- Expiry of the kit is stated on labels.

7. TECHNICAL HINTS AND LIMITATIONS

- Do not combine leftover reagents with those reserved for additional wells.
- It is recommended that all standards, controls and samples be run in duplicate.
- Samples that are >20 ng/ml should be diluted with ELISA Buffer 1X.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate forcefully on absorbent paper.
- Crystals could appear in the 10X solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
- Once reagents have been added to the 16-well strips, DO NOT let the strips DRY at any time during the assay.
- The Stop Solution (STOP) consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.
- To reduce interference from rheumatoid factor 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).

8. GENERAL ELISA PROTOCOL

8.1. Preparation and Storage of Reagents

Prepare just the appropriate amount of the buffers necessary for the assay!

- a. The **Wash Buffer 10X** has to be diluted with deionized or distilled water 1:10 before use (eg 10 ml Wash Buffer 10X + 90 ml water) to obtain Wash Buffer 1X.
- b. The **ELISA Buffer 10X** has to be diluted with deionized or distilled water 1:10 before use (eg 10 ml ELISA Buffer 10X + 90 ml water) to obtain ELISA Buffer 1X.
- c. The **mouse BAFF Standard (STD)** has to be reconstituted with 100 μ l of ELISA Buffer 1X.
 - This reconstitution produces a stock solution of **100 μ g/ml**. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.
! The reconstituted standard is aliquoted and stored at -20°C ! Avoid freeze/ thaw cycles!
 - Dilute the standard protein concentrate (STD) (100 μ g/ml) in ELISA Buffer 1X. A seven-point standard curve using 2-fold serial dilutions in ELISA Buffer 1X is recommended.
 - Suggested standard points are 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml and 0 ng/ml.
 - Start with the dilution of the concentrate (STD):

To obtain	Add	Into
10 μ g/ml	5 μ l mBAFF (STD)(100 μ g/ml)	45 μ l ELISA Buffer 1X
100 ng/ml	5 μ l mBAFF (STD)(10 μ g/ml)	495 μ l ELISA Buffer 1X

- Dilute further for the standard curve:

To obtain	Add	Into
20 ng/ml	150 μ l mBAFF (100 ng/ml)	600 μ l ELISA Buffer 1X
10 ng/ml	300 μ l mBAFF (20 ng/ml)	300 μ l ELISA Buffer 1X
5 ng/ml	300 μ l mBAFF (10 ng/ml)	300 μ l ELISA Buffer 1X
2.5 ng/ml	300 μ l mBAFF (5 ng/ml)	300 μ l ELISA Buffer 1X
1.25 ng/ml	300 μ l mBAFF (2.5 ng/ml)	300 μ l ELISA Buffer 1X
0.625 ng/ml	300 μ l mBAFF (1.25 ng/ml)	300 μ l ELISA Buffer 1X
0.3125 ng/ml	300 μ l mBAFF (0.625 ng/ml)	300 μ l ELISA Buffer 1X
0 ng/ml	300 μ l ELISA Buffer 1X	Empty tube

- d. **Detection Antibody (AB)** has to be diluted 1:1000 in Diluent Buffer (eg 10 μ l AB + 10 ml Diluent Buffer). The diluted Detection Antibody is not stable and cannot be stored.
- e. **Horseradish peroxidase (HRP) Labelled Streptavidin (STREP-HRP)** has to be reconstituted with 100 μ l ELISA Buffer 1X. **The reconstituted STREP-HRP is stable for one month at 2-8°C!** Dilute the STRE-HRP to the working concentration by adding 50 μ l in 10 ml of ELISA Buffer 1X. The diluted STREP-HRP is not stable and cannot be stored.
- f. **Serum or Cell Culture Supernatants** have to be diluted in ELISA Buffer 1X.
- g. **All other reagents** are ready to use and are stable until the expiry date when stored at 2-8°C

8.2. Assay procedure

1. Determine the number of 16-well strips needed for the assay and insert them in the frame for current use. The extra strips are left in the bag with 2 silica gel minibags and are stable for one month when stored at 2-8°C.

2. Add 300 μ l of Wash Buffer (Wash Buffer 1X) using a multichannel pipette or autowasher. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.

3. Add 100 μ l of the different standards (**see 8.1. Preparation and Storage of Reagents, section c. mouse BAFF Standard (STD)**) into the appropriate wells in duplicate! At the same time, add 100 μ l of diluted serum or cell culture supernatant samples in duplicate to the wells .

4. Cover the plate with plastic film and incubate for **1 hour at room temperature (RT°C)**.

5. Aspirate the coated wells and add 300 μ l of Wash Buffer (Wash Buffer 1X) using a multichannel pipette or autowasher. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.

6. Add 100 μ l to each well of the diluted Detection Antibody (**see 8.1 Preparation and Storage of Reagents, section d. Detection Antibody (AB)**).

7. Cover the plate with plastic film and incubate for **1 hour at RT°C**.

8. Aspirate the coated wells and add 300 μ l of Wash Buffer (Wash Buffer 1X) using a multichannel pipette or autowasher. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.

9. Add 100 μ l to each well of the diluted HRP Labelled Streptavidin (see 8.1. Preparation and Storage of Reagents, section e. STREP-HRP)

10. Cover the plate with plastic film and incubate for 30 min at RT°C.

11. Aspirate the coated wells and add 300 μ l of Wash Buffer (Wash Buffer 1X) using a multichannel pipette or autowasher. Repeat the process for a total of three washes. After the last wash, complete removal of liquid is essential for good performance.

12. Add 100 μ l to each well of TMB substrate solution (TMB).

13. Allow the color reaction to develop **in the dark for 15-30 min at RT°C**. Do not cover the plate.

14. Stop the reaction by adding 50 μ l of Stop Solution (**STOP**). Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution (**STOP**) is added.

! CAUTION: CORROSIVE SOLUTION!

15. Measure the OD at 450 nm in an ELISA reader.

9. CALCULATION OF RESULTS

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 ng/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the vertical (Y) axis vs. the corresponding mBAFF concentration (ng/ml) on the horizontal axis (see **10. PERFORMANCE CHARACTERISTICS**).
- Calculate results using graph paper or curve-fitting statistical software. 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. PERFORMANCE CHARACTERISTICS

- a. **Expected values.** The levels of BAFF in the serum of mouse were evaluated and ranged from 2.7 ng/ml to 7.1 ng/ml. The mean value was 4.6 ng/ml.
- b. **Sensitivity.** The lowest level of BAFF (mouse) that can be detected by this assay is 0.2 ng/ml.
- c. **Assay range.** 0.312 ng/ml - 20 ng/ml
- d. **Specificity.** The monoclonal antibodies used in this ELISA are specific for the measurement of natural and recombinant mouse BAFF. They do not cross-react with human BAFF.
- e. **Precision /Reproducibility.**
 - Intra-assay precision: (CV < 7%)

samples	Mean (ng/ml)	SD	CV %	n
1	3.749	0.244	6.51	20
2	3.813	0.227	5.95	20

- Inter-assay precision (CV: <10%):

samples	Mean (ng/ml)	SD	CV %	n
1	275, 34	4.50	1.63	4
2	68.66	6,25	9.10	4

- f. **Recovery.** The recovery of mBAFF added to serum averages 87%.

g. **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

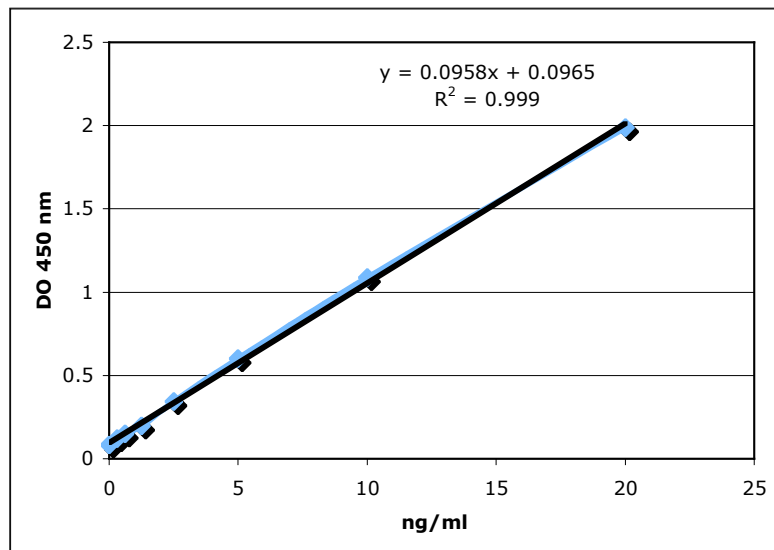


Figure: Standard curve example.



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