BioVendor Group

ENG

Instructions for Use:

MOUSE BAFF SOLUBLE ELISA

Catalogue number:

RAG032R

For research use only.

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
	ENG.001.A
New edition	

1. INTENDED USE

The BAFF, soluble (mouse) ELISA kit is to be used for the in vitro quantitative determination of mouse BAFF in serum, plasma and cell culture supernatant. This ELISA Kit is for research use only.

2. STORAGE, EXPIRATION

- Reagent must be stored at 2-8°C when not in use
- Plate and reagents should be at room temperature before use.
- Do not expose reagents to temperatures greater than 25°C.

3. INTRODUCTION

BAFF (B cell activation factor of the TNF family, also known as CD257, BLyS or TALL1) is a key survival factor for peripheral B cells. BAFF is a homotrimeric type II transmembrane protein that can be proteolytically processed by furin to be released as soluble cytokine [1]. Soluble BAFF adopts the classical trimeric form of the TNF-family ligand. However, BAFF has the unique property among the TNF-ligand to assemble as a 60-mer [2]. BAFF is mainly produced by innate immune cells such as neutrophils, monocytes, macrophages, dendritic cells, follicular dendritic cells. T cells, activated B cells, some malignant B cells and also non-lymphoid cells like astrocytes, synoviocytes and epithelial cells can also produce BAFF. 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 together with IL-6, promotes Ig class-switching and plasma cell differentiation [1]. Besides its major role in B cell biology, BAFF co-stimulates activated T cells. In humans, elevated levels of soluble BAFF have been detected in the serum of patients with various autoimmune diseases [3], such as Sjögren's syndrome [4], Rheumatoid Arthritis (RA) [5], Multiple sclerosis (MS) [6] and Systemic Lupus Erythematosus (SLE) [7]. BAFF is also increased levels in some lymphoid cancers [8]. Deregulated expression of BAFF leads to autoimmune disorders in mice [9].

4. TEST PRINCIPLE

This assay is a sandwich Enzyme Linked-Immunosorbent Assay (ELISA) for quantitative determination of mouse BAFF in serum, plasma and cell culture supernatant. A monoclonal antibody specific for mouse BAFF has been precoated onto the 96-well microtiter plate. Standards (STD) and samples are pipetted into the wells for binding to the coated antibody. After extensive washing to remove unbound compounds, mouse BAFF is recognized by the addition of a biotinylated monoclonal antibody specific for mouse BAFF (DET). After removal of excess biotinylated antibody, streptavidin-peroxidase (STREP-HRP) 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 mouse BAFF in the samples.

5. TECHNICAL HINTS

- It is recommended that all standards, controls and samples be run in duplicate.
- Do not combine leftover reagents with those reserved for additional wells.
- Reagents from the kit with a volume less than 100µl should be centrifuged.
- Residual wash liquid should be drained from the wells after last wash by tapping the plate 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.
- Keep TMB Solution protected from light.
- The Stop Solution consists of sulfuric acid. Although diluted, the Stop Solution should be handled with gloves, eye protection and protective clothing.

6. REAGENT SUPPLIED

Kit Components	Quantity
1 vial mouse BAFF Standard (lyophilized)	100 ng
1 vial Detection Antibody	30 µl
1 vial HRP Labeled Streptavidin (lyophilized)	2 µg
2 bottles Wash Buffer 10X	2 x 30 ml
1 bottle ELISA Buffer 10X	1 x 30 ml
1 bottle TMB Substrate Solution	12 ml
1 bottle Stop Solution	12 ml
1 plate coated with mBAFF Antibody	6 x 16-well strips
2 plate Covers (plastic film)	
2 silica Gel Minibags	

7. MATERIAL REQUIRED BUT NOT SUPPLIED

- Microtiterplate reader at 450 nm
- Calibrated precision pipettes. Disposable pipette tips
- Deionized water
- Microtubes or equivalent for preparing dilutions
- Disposable plastic containers for preparing working buffers
- Plate washer: automated or manual
- Glass or plastic tubes for diluting and aliquoting standard

8. PREPARATION OF REAGENTS

NOTE: Prepare just the appropriate amount of the buffers necessary for the assay.

8.1 Wash Buffer 10X

has to be diluted with deionized water 1:10 before use (e.g. 30 ml Wash Buffer 10X + 270 ml water) to obtain Wash Buffer 1X.

8.2 ELISA Buffer 10X

has to be diluted with deionized water 1:10 before use (e.g. 10 ml ELISA Buffer 10X + 90 ml water) to obtain ELISA Buffer 1X.

8.3 Detection Antibody

has to be diluted to 1:500 in ELISA Buffer 1X (4 µl AB + 2 ml ELISA Buffer 1X).
NOTE: The diluted Detection Antibody is not stable and cannot be stored!

8.4 HRP Labeled Streptavidin

has to be reconstituted with 100 µl of ELISA Buffer 1X.

- After reconstitution of STREP-HRP, prepare aliquots and store them at -20°C. **Avoid freeze/thaw cycles**.
- Dilute the reconstituted STREP-HRP to the working concentration by adding 50 µl in 10 ml of ELISA Buffer 1X (1:200).

NOTE: The diluted STREP-HRP is not stable and cannot be stored!

8.5 Mouse BAFF Standard

has to be reconstituted with 100 µl of ELISA Buffer 1X

- This reconstitution produces a stock solution of 1 µg/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.

NOTE: The reconstituted standard is aliquoted and stored at -20°C!

- Dilute the standard protein concentrate (STD) (**1 µg/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:
  1000, 500, 250, 125, 62.5, 31.2, 15.6 and 0 pg/ml.

Start with the dilution of the concentrate (STD):

To obtain	Add	Into
50 ng/ml	20 µl of BAFF (STD) (1 µg/ml)	380 µl of ELISA Buffer 1X

Dilute further for the standard curve:

To obtain	Add	Into
1000 pg/ml	20 µl of BAFF (50 ng/ml)	980 µl of ELISA Buffer 1X
500 pg/ml	300 µl of BAFF (1000 pg/ml)	300 µl of ELISA Buffer 1X
250 pg/ml	300 µl of BAFF (500 pg/ml)	300 µl of ELISA Buffer 1X
125 pg/ml	300 µl of BAFF (250 pg/ml)	300 µl of ELISA Buffer 1X
62.5 pg/ml	300 µl of BAFF (125 pg/ml)	300 µl of ELISA Buffer 1X
31.2 pg/ml	300 µl of BAFF (62.5 pg/ml)	300 µl of ELISA Buffer 1X
15.6 pg/ml	300 µl of BAFF (31.2pg/ml)	300 µl of ELISA Buffer 1X
0 pg/ml	300 µl of ELISA Buffer 1X	Empty tube

9. PREPARATION OF SAMPLES

9.1 Serum

Use a serum separator tube. Let samples clot at room temperature for 30 minutes before centrifugation for 20 minutes at 1,000xg. Assay freshly prepared serum or store serum in aliquot at - 20°C for later use. Avoid repeated freeze/thaw cycles.

9.2 Plasma

Collect plasma using heparin, EDTA, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000xg within 30 minutes of collection. Assay freshly prepared plasma or store plasma sample in aliquot at - 20°C for later use. Avoid repeated freeze/ thaw cycles.

9.3 Serum, Plasma or Cell Culture Supernatant

have to be diluted in ELISA Buffer 1X. Samples containing visible precipitates must be clarified before use.
NOTE: As a starting point, 1/10 to 1/20 dilutions of serum or plasma are recommended! If sample values fall outside the detection range of the assay, a lower or higher dilution may be required!

10. 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 stored at 4°C.
   NOTE: Remaining 16-well strips coated with mouse BAFF antibody when opened can be stored in the presence of 2 silica gel minibags at 4°C for up to 1 month.

2. Add 100 µl of the different standards into the appropriate wells in duplicate! At the same time, add 100 µl of diluted serum, plasma or cell culture supernatant samples in duplicate to the wells **(see Preparation of Reagents and Preparation of Samples).**

3. Cover the plate with plastic film and incubate for **2 hours at room temperature** (RT).

4. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.

5. Add 100 µl to each well of the diluted Detection Antibody **(see Preparation of Reagents)**.

6. Cover the plate with plastic film and incubate for **1 hour at room temperature**.

7. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.

8. Add 100 µl to each well of the diluted HRP Labeled Streptavidin **(see Preparation of Reagents)**.

9. Cover the plate with plastic film and incubate for **30 min at room temperature**.

10. Aspirate the coated wells and add 300 µl of Wash Buffer 1X using a multichannel pipette or auto-washer. Repeat the process for a total of five washes. After the last wash, complete removal of liquid is essential for good performance.

11. Add 100 µl to each well of TMB substrate solution.

12. Allow the color reaction to develop **at room temperature (RT) in the dark for 8-15 minutes**. Do not cover the plate.

13. Stop the reaction by adding 100 µl of Stop Solution. Tap the plate gently to ensure thorough mixing. The substrate reaction yields a blue solution that turns yellow when Stop Solution is added.
    CAUTION: CORROSIVE SOLUTION !

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

11. CALCULATIONS

- Average the duplicate readings for each standard, control and sample and subtract the average blank value (obtained with the 0 pg/ml point).
- Generate the standard curve by plotting the average absorbance obtained for each standard concentration on the horizontal (X) axis vs. the corresponding BAFF concentration (pg/ml) on the vertical axis (see chapter TYPICAL DATA).
- Calculate the BAFF concentrations of samples by interpolation of the regression curve formula as shown above in a form of a quadratic equation
- If the test sample was diluted, multiply the interpolated value by the dilution factor to calculate the concentration of mouse BAFF in the sample.

12. PERFORMANCE CHARACTERISTICS

12.1 Sensitivity (Limit of detection)

The lowest level of mouse BAFF that can be detected by this assay is 14 pg/ml.
NOTE: The Limit of detection was measured by adding two standard deviations to the mean value of 50 zero standard.

12.2 Assay range

15.6 pg/ml – 1000 pg/ml

12.3 Specificity:

This ELISA is specific for the measurement of natural and recombinant mouse BAFF. It does not cross-react with human BAFF.

12.4 Intra-assay precision:

Four samples of known concentrations of mouse BAFF were assayed in replicates 4 times to test precision within an assay.

Samples	Means (ng/ml)	SD	CV (%)	n
A1	0.99	0.021	2.12	4
A2	0.62	0.013	2.01	4
A3	0.5	0.025	4.70	4
A4	1.87	0.122	6.53	4

12.5 Inter-assay precision:

Four samples of known concentrations of mouse BAFF were assayed in 4 separate assays to test precision between assays.

Samples	Means (ng/ml)	SD	CV (%)	n
B1	0.98	0.019	1.91	4
B2	1.88	0.110	5.85	4
B3	1.90	0.031	1.67	4
B4	2.69	0.186	6.90	4

12.6 Recovery:

When samples (serum) are spiked with known concentrations of mouse BAFF, the spiking recovery ranged from 103% to 115% (average 108%).

12.7 Linearity:

Different mouse serum samples containing BAFF were diluted several fold (1/10 to 1/20) and the measured recoveries ranged from 92% to 110% (average 107%).

12.8 Expected values:

BAFF levels range in mouse plasma and serum from **0.5 to >5 ng /ml**.

13. TYPICAL DATA

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

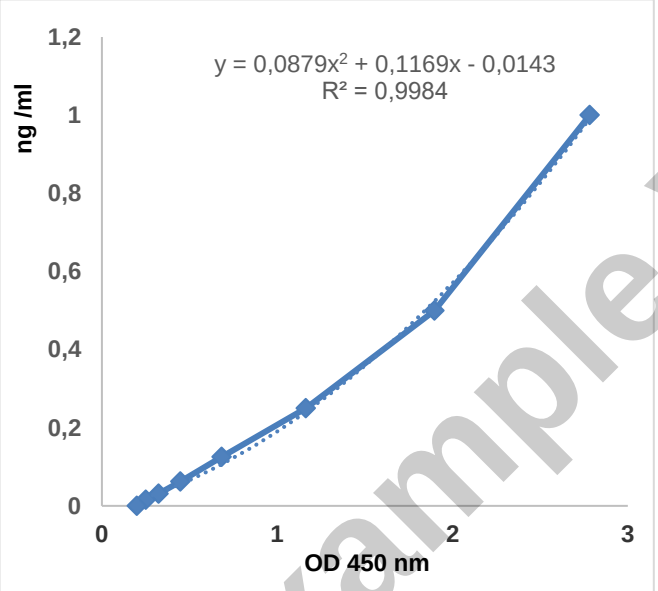


Figure: Standard curve

Standard mBAFF (pg/ml)	Optical Density (mean)
1000	2.561
500	1.716
250	1.062
125	0.696
62.5	0.489
31.2	0.385
15.6	0.326
0	0.255

14. TROUBLESHOOTING AND FAQS

PROBLEM	POSSIBLE CAUSES	SOLUTIONS
No signal or weak signal	Omission of key reagent	Check that all reagents have been added in the correct order.
	Washes too stringent	Use an automated plate washer if possible.
	Incubation times inadequate	Incubation times should be followed as indicated in the manual.
	Plate reader settings not optimal	Verify the wavelength and filter setting in the plate reader.
	Incorrect assay temperature	Use recommended incubation temperature. Bring substrates to room temperature before use.
High background	Concentration of STREP-HRP too high	Use recommended dilution factor.
	Inadequate washing	Ensure all wells are filling wash buffer and are aspirated completely.
Poor standard curve	Wells not completely aspirated	Completely aspirate wells between steps.
	Reagents poorly mixed	Be sure that reagents are thoroughly mixed.
Unexpected results	Omission of reagents	Be sure that reagents were prepared correctly and added in the correct order.
	Dilution error	Check pipetting technique and double-check calculations.

15. REFERENCES

1 Cracking the BAFF code: F. Mackay & P. Schneider; Nat. Rev. Immunol. 9, 491 (2009)

2 TACI, unlike BAFF-R, is solely activated by oligomeric BAFF and APRIL to support survival of activated B cells and plasmablasts: C. Bossen, et al.; Blood 111, 1004 (2009)

3 BAFF: a local and systemic target in autoimmune diseases: I. Moisini & A. Davidson; Clin. Exp. Immunol. 158, 155 (2009)

4 B-cell tolerance breakdown in Sjögren's syndrome: focus on BAFF: M.M. Varin, et al.; Autoimmun. Rev. 9, 604 (2010)

5 Concentrations of BAFF correlate with autoantibody levels, clinical disease activity, and response to treatment in early rheumatoid arthritis: S. Bosello, et al.; J. Rheumatol. 35, 1256 (2008)

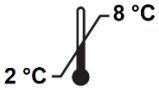
6 A BAFF antagonist suppresses experimental autoimmune encephalomyelitis by targeting cell-mediated and humoral immune responses: N.D. Huntington, et al.; Int. Immunol. 18, 1473 (2006)

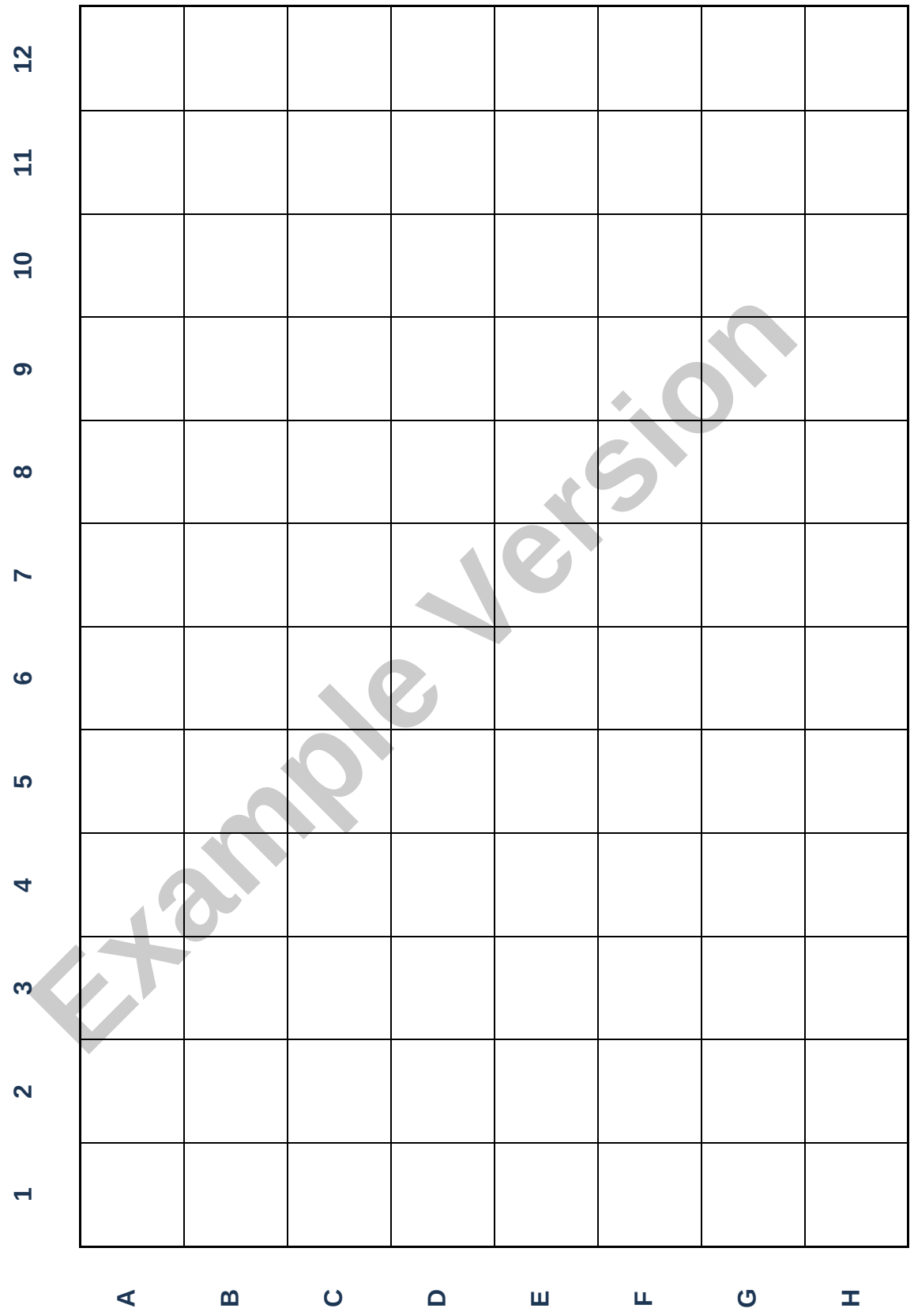
7 B lymphocyte stimulator overexpression in patients with systemic lupus erythematosus: longitudinal observations: W. Stohl, et al.; Arthritis Rheum. 48, 3475 (2003)

8 Serum BAFF predicts prognosis better than APRIL in diffuse large B-cell lymphoma patients treated with rituximab plus CHOP chemotherapy: S.J. Kim, et al.; Eur. J. Haematol. 81, 177 (2008)

9 Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren’s syndrome: J.Groom, et al.; J Clinical Invest., 109, 59 (2002)

16. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



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