Quantikine® ELISA

Mouse BAFF/BLyS/TNFSF13B Immunoassay

Catalog Number MBLYS0

For the quantitative determination of mouse B cell Activating Factor (BAFF) concentrations in cell culture supernates, serum, and plasma.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

B-cell activating factor (BAFF), also known as BLyS, TALL-1, and THANK, is a TNF superfamily member (TNFSF13B) best known for its role in the survival and maturation of B cells (1-3). Mouse BAFF is 32 kDa type II transmembrane protein (4), and an 18 kDa form can also be shed into the serum (5-9). The N-terminal side of the mouse BAFF TNF homology domain contains a furin cleavage site (RNRR) responsible for the release of soluble BAFF (4). A conserved alternatively spliced isoform termed BAFF has also been described (10). It can form heteromultimers with BAFF and may act to negatively regulate BAFF secretion (10). BAFF is produced by several cell types and tissues including monocytes, macrophages, neutrophils, dendritic cells, T lymphocytes, spleen, lymph node, and bone marrow (4, 5, 11, 12). It is thought to exist as a homotrimer, but it may also exist as a heteromer in association with related TNFSF member APRIL (13, 14).

BAFF is a ligand for at least three TNF receptor superfamily (TNFRSF) members: B-cell maturation antigen (BCMA/TNFRSF17), transmembrane activator and calcium-modulator and cyclophilin ligand interactor (TACI/TNFRSF13B), and BAFF receptor (BAFF R/BR3/ TNFRSF13C) (15-21). These receptors are putative type III proteins that lack a signal sequence (22, 23). Whereas TACI and BCMA are receptors for both BAFF and APRIL, BAFF R selectively binds BAFF (21). TACI and BAFF R are cell surface receptors, and although BCMA can be found at the plasma membrane as well, significant expression is also localized to perinuclear Golgi-like structures (21, 23-25). All three receptors are primarily expressed by B cells (3, 16, 19, 21, 26).

Studies utilizing genetically modified mice provide strong evidence that BAFF plays a major role in B cell survival and maturation. BAFF knockout mice exhibit a loss of follicular and marginal zone B cells in lymph node and spleen, while bone marrow cells and B1 cells of the peritoneum are generally unaffected (27, 28). A similar phenotype is observed in A/WySnJ mice, a strain that exhibits a mutation in a portion of the BAFF R gene encoding the signaling domain of the receptor (21). BAFF appears to be necessary for the proper transition from T1 to T2 phases of the B cell maturation pathway (27, 28). Mechanisms underlying BAFF effects on B cell survival may include the upregulation or downregulation of anti- or pro-apoptotic members of the Bcl-2 family, respectively (29-34). Over expressing BAFF transgenic mice exhibit elevated B cell numbers in spleen and lymph node (29, 31, 35). This is accompanied by expanded follicles and increases in the number and size of germinal centers (29, 35). These mice also exhibit characteristics of autoimmune disease including elevated levels of auto-antibodies, immunoglobulin deposits in the kidneys, and glomerulonephritis accompanied by kidney dysfunction (29, 35). It is suggested that BAFF transgenic mice exhibit characteristics similar to those found in patients with systemic lupus erythematosus (SLE) (35).Consistent with a role in human autoimmune disorders, BAFF is elevated in the serum of patients with SLE and Sjögren's syndrome (5, 7, 8). It is also produced locally in the joints of patients with inflammatory arthritis and serum levels correlate with antibody titers in arthritis and Sjögren's syndrome (6, 8, 36). Consequently, BAFF may act as a potential target for autoimmune therapy (37).

The Quantikine® Mouse BAFF/BLyS/TNFSF13B Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse BAFF/BLyS in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse BAFF/BLyS and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse BAFF/BLyS showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse BAFF/BLyS.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse BAFF/BLyS has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any BAFF/BLyS present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse BAFF/BLyS is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of BAFF/BLyS bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- For best results, pipette reagents and samples into the center of each well.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse BAFF/BLyS Microplate	893143	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse BAFF/BLyS.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse BAFF/BLyS Standard	893145	Recombinant mouse BAFF/BLyS in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Mouse BAFF/BLyS Control	893146	Recombinant mouse BAFF/BLyS in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse BAFF/BLyS Conjugate	893144	21 mL of a polyclonal antibody specific for mouse BAFF/BLyS conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1N	895488	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-12	895214	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

PRECAUTIONS

Assay Diluent RD1N contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require at least a 3-fold dilution and may require up to a 10-fold dilution prior to assay. A suggested 3-fold dilution is 50 µL of sample + 100 µL of Calibrator Diluent RD6-12.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

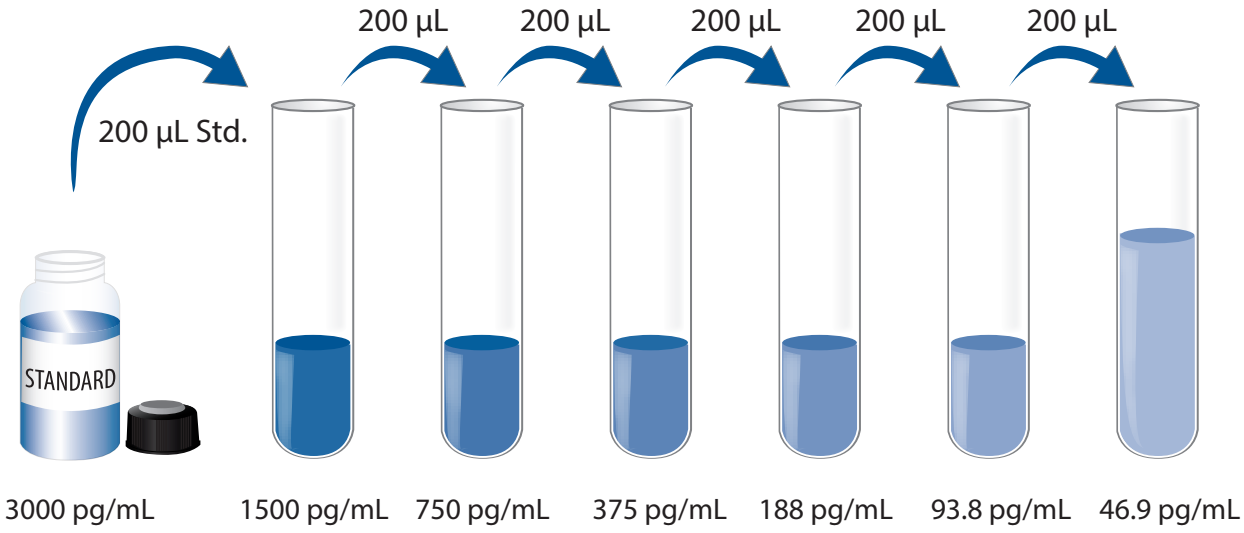
Mouse BAFF/BLyS Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 120 µL of the resultant mixture is required per well.

Mouse BAFF/BLyS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse BAFF/BLyS Standard with Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 3000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD6-12 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse BAFF/BLyS Standard (3000 pg/mL) serves as the high standard. Calibrator Diluent RD6-12 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 80 µL of Assay Diluent RD1N to each well.
4. Add 40 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 120 µL of Mouse BAFF/BLyS Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 120 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 120 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

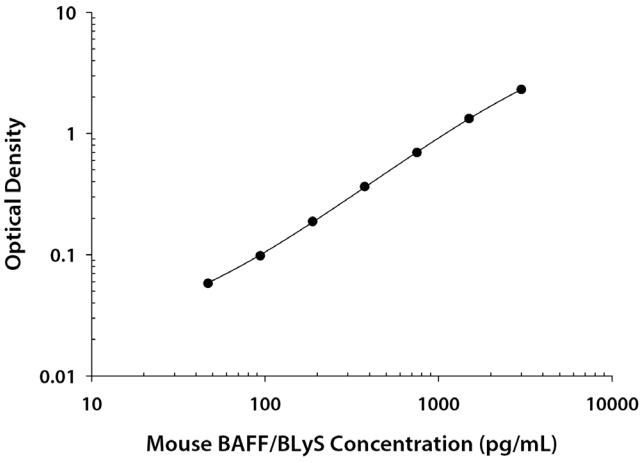
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse BAFF/BLyS concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.022	0.022	—
46.9	0.078 0.081	0.080	0.058
93.8	0.119 0.122	0.120	0.098
188	0.207 0.212	0.210	0.188
375	0.386 0.387	0.386	0.364
750	0.706 0.729	0.718	0.696
1500	1.348 1.349	1.348	1.326
3000	2.307 2.350	2.328	2.306

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	50	64	56
Mean (pg/mL)	125	288	1315	127	325	1262
Standard deviation	3.6	7.0	30.3	16.0	27.0	136
CV (%)	2.9	2.4	2.3	12.6	8.3	10.8

RECOVERY

The recovery of mouse BAFF/BLyS spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	103	90-108%
Serum* (n=6)	98	86-115%
EDTA plasma* (n=6)	96	85-103%
Heparin plasma* (n=6)	94	85-100%

*Samples were spiked and then diluted 10-fold prior to assay.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse BAFF/BLyS in each matrix were diluted with calibrator diluent and assayed.

		Cell culture supernates (n=5)	Serum* (n=6)	EDTA plasma* (n=6)	Heparin plasma* (n=6)
1:2	Average % of Expected	98	101	98	102
	Range (%)	96-99	96-103	95-102	97-103
1:4	Average % of Expected	93	101	101	102
	Range (%)	84-98	99-104	97-105	96-105
1:8	Average % of Expected	87	102	100	102
	Range (%)	80-93	98-106	94-106	96-108
1:16	Average % of Expected	———	100	102	101
	Range (%)	———	95-104	94-108	96-110

*Samples were diluted prior to assay.

SENSITIVITY

Forty-one assays were evaluated and the minimum detectable dose (MDD) of mouse BAFF/BLyS ranged from 1.8-7.8 pg/mL. The mean MDD was 4.3 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse BAFF/BLyS produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse BAFF/BLyS in this assay. Serum and plasma samples are not matched.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	6482	4562-13,768	2071
EDTA plasma (n=20)	5552	3829-8662	1291
Heparin plasma (n=20)	4647	3100-6119	797

Cell Culture Supernates:
Spleens from four mice were homogenized and seeded into 60 mL of RPMI supplemented with 10% fetal bovine serum, 10 ng/mL of recombinant human IL-2, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 24 hours. The cell culture supernate was removed, assayed for mouse BAFF/BLyS, and measured 671 pg/mL.

RAW264.7 mouse monocyte/macrophage cells were cultured in DMEM supplemented with 10% fetal bovine serum. Cells were seeded at 5 x 10^4 cells/mL unstimulated or stimulated with 2.5 ng/mL LPS and 10 ng/mL recombinant mouse IL-10. On day 3, the media was removed and fresh media was added. The media was collected on day 7.

Condition	(pg/mL)
Unstimulated	274
Stimulated	434

SPECIFICITY

This assay recognizes natural and recombinant mouse BAFF/BLyS.

The factors listed below were prepared at 60 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 60 ng/mL in a mid-range mouse BAFF/BLyS control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

4-1BB
4-1BB Ligand
BCMA
CD30
CD30 Ligand/His
CD40
CD40 Ligand
EDA/His
EDAR
Fas
Fas Ligand
Lymphotoxin α1/β2
Lymphotoxin α2/β1
NGF R
OPG
OX40
OX40 Ligand
RANK
TNF-α
TNF-α (short)
TNF RI
TNF RII
TROY
TRAIL R2
TRANCE
TWEAK R

Recombinant human:

APRIL
BAFF
BAFF R
CD27
DcR3
DR3
DR6
EDA-A2
GITR
GITR Ligand
HVEM
LIGHT
LTβ R
RELT
TL1A
TNF-β
TRAIL
TRAIL R1
TRAIL R3
TRAIL R4
TWEAK
TWEAK R
XEDAR

Recombinant mouse BAFF R and recombinant mouse TACI interfere at concentrations > 7.5 ng/mL and 30 ng/mL, respectively.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.