

Quantikine[®]

Human BAFF/BLyS/TNFSF13B Immunoassay

Catalog Number DBLYS0
SBLYS0
PDBLYS0

For the quantitative determination of human B Cell Activating Factor Belonging to the TNF Family (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). The BAFF gene encodes a putative 285 amino acid (aa) type II transmembrane protein (4). A 152 aa form can also be shed from the membrane and is detectable in human serum (4 - 8). The N-terminal side of the human BAFF TNF homology domain contains a furin cleavage site (RNKR) responsible for the release of soluble BAFF (9). A conserved alternatively spliced isoform termed Δ 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, 9, 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 Human BAFF Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human BAFF in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human BAFF and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human BAFF showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Human BAFF kit can be used to determine relative mass values for naturally occurring human BAFF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for BAFF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BAFF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for BAFF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of BAFF bound in the initial step. The color development is stopped and the intensity of the color is measured.

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, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Description	Part #	Cat. # DBLYS0	Cat. # SBLYS0
BAFF/BLyS Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against BAFF.	892288	1 plate	6 plates
BAFF/BLyS Conjugate - 21 mL/vial of polyclonal antibody against BAFF conjugated to horseradish peroxidase with preservatives.	892289	1 vial	6 vials
BAFF/BLyS Standard - Recombinant human BAFF in a buffer with preservatives; lyophilized.	892290	1 vial	6 vials
Assay Diluent RD1-72 - 11 mL/vial of a buffer with preservatives.	895367	1 vial	6 vials
Calibrator Diluent RD5K - 21 mL/vial of a buffered protein base with preservatives. <i>For cell culture supernate samples.</i>	895119	1 vial	6 vials
Calibrator Diluent RD6Q - 21 mL/vial of animal serum with preservatives. <i>For serum/plasma samples.</i>	895128	1 vial	6 vials
Wash Buffer Concentrate - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
Color Reagent A - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
Color Reagent B - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
Stop Solution - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
Plate Covers - Adhesive strips.	—	4 strips	24 strips

DBLYS0 contains sufficient materials to run an ELISA on one 96 well plate.

SBLYS0 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDBLYS0). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

STORAGE

Unopened Kit	Store at 2 - 8° C. Do not use past kit expiration date.	
Opened/ Reconstituted Reagents	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1-72	
	Calibrator Diluent RD5K	
	Calibrator Diluent RD6Q	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

*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.
- Test tubes for dilution.
- Human BAFF/BLyS Controls (optional; available from R&D Systems).

PRECAUTIONS

Calibrator Diluent RD6Q 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. Wear eye, hand, face, and clothing protection when using this material.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at $\leq -20^{\circ}$ C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}$ 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 2-fold dilution and may require up to a 10-fold dilution. A suggested 2-fold dilution is 100 μ L sample + 100 μ L Calibrator Diluent RD6Q.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

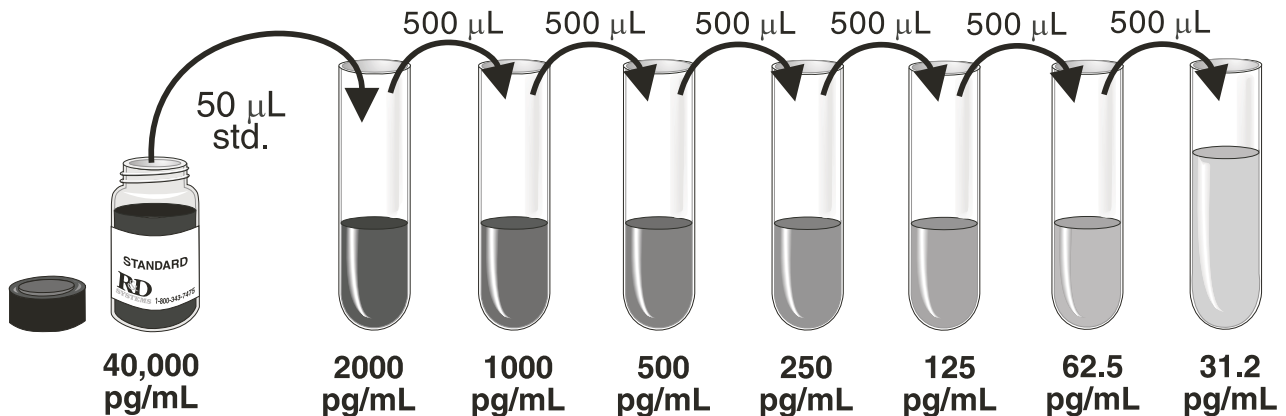
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 μ L of the resultant mixture is required per well.

BAFF/BLyS Standard - **Refer to vial label for reconstitution volume.** Reconstitute the BAFF/BLyS Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/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.

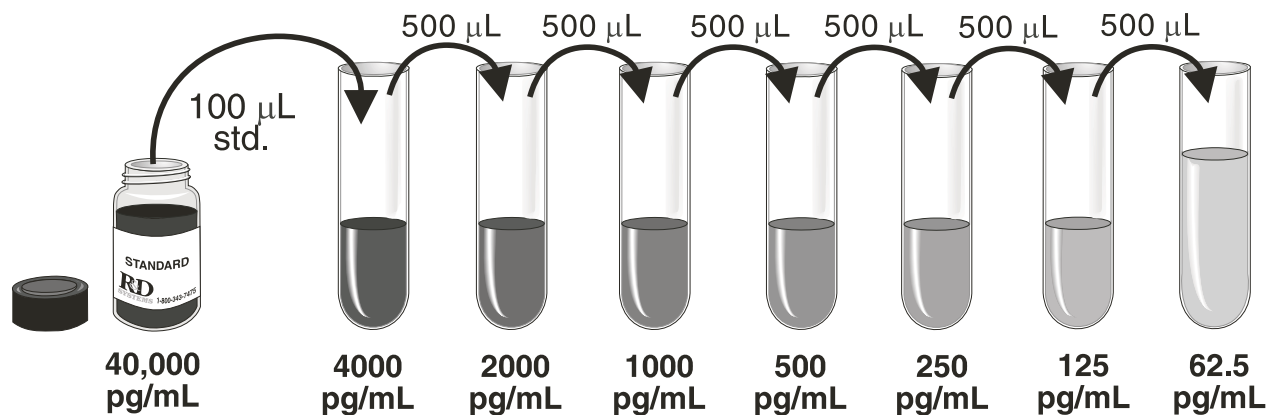
For Cell Culture Supernate samples:

Pipette 950 μL of Calibrator Diluent RD5K into the 2000 pg/mL tube. Pipette 500 μL of Calibrator Diluent RD5K into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 pg/mL).



For Serum/Plasma samples:

Pipette 900 μL of Calibrator Diluent RD6Q into the 4000 pg/mL tube. Pipette 500 μL of Calibrator Diluent RD6Q into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. Calibrator Diluent RD6Q 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 samples, controls, and standards be assayed in duplicate.

1. Prepare all reagents, working standards, 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 100 μL of Assay Diluent RD1-72 to each well.
4. **For serum/plasma samples** - Add 50 μL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
For cell culture supernate samples - Add 75 μL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 μL of BAFF/BLyS Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 μL of Substrate Solution to each well. **Protect from light.**
For serum/plasma samples - Incubate for 30 minutes at room temperature.
For cell culture supernate samples - Incubate for 20 minutes at room temperature.
9. Add 50 μL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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.

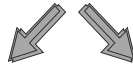
*Serum/plasma samples require dilution. See Sample Preparation section.

ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Add 100 μL Assay Diluent RD1-72 to each well.



3. Serum/Plasma Samples:

Add 50 μL Standard, control, or sample* to each well.
Incubate 2 hours at RT.

Cell Culture Supernate Samples:

Add 75 μL Standard, control, or sample to each well.
Incubate 2 hours at RT.



4. Aspirate and wash 4 times.



5. Add 200 μL Conjugate to each well.
Incubate 2 hours at RT.



6. Aspirate and wash 4 times.



7. Add 200 μL Substrate Solution to each well. **Protect from light.**

Serum/Plasma Samples:

Incubate 30 minutes at RT.



Cell Culture Supernate Samples:

Incubate 20 minutes at RT.



8. Add 50 μL Stop Solution to each well.
Read at 450 nm within 30 minutes.
 λ correction 540 or 570 nm.

*Serum/plasma samples require dilution.
See Sample Preparation section.

CALCULATION OF RESULTS

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

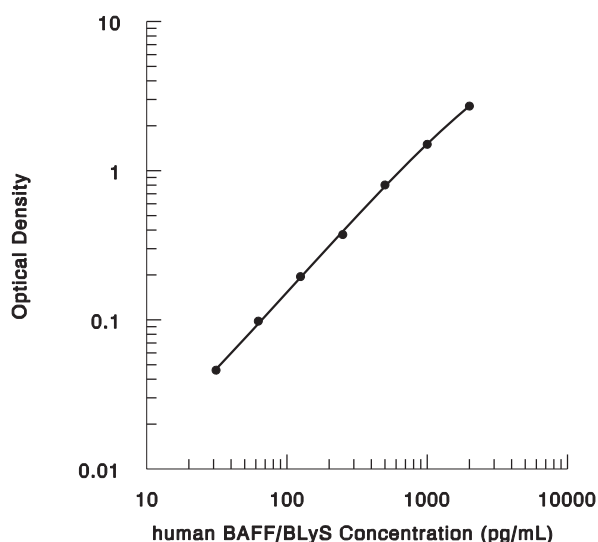
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the BAFF 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

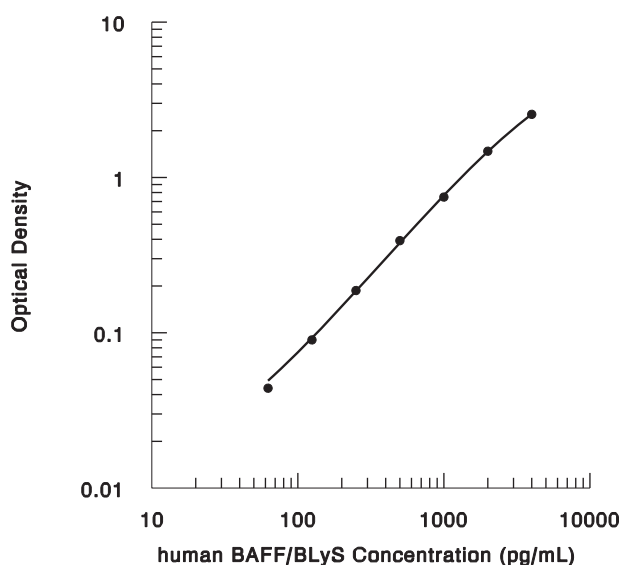
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Calibrator Diluent RD5K



pg/mL	O.D.	Average	Corrected
0	0.020 0.022 0.065	0.021	—
31.2	0.069 0.114	0.067	0.046
62.5	0.123 0.210	0.119	0.098
125	0.222 0.391	0.216	0.195
250	0.396 0.796	0.394	0.373
500	0.849 1.476	0.823	0.802
1000	1.571 2.671	1.524	1.503
2000	2.791	2.731	2.710

Calibrator Diluent RD6Q



pg/mL	O.D.	Average	Corrected
0	0.016 0.016 0.060	0.016	—
62.5	0.060 0.105	0.060	0.044
125	0.107 0.200	0.106	0.090
250	0.205 0.405	0.203	0.187
500	0.410 0.738	0.408	0.392
1000	0.790 1.467	0.764	0.748
2000	1.519 2.566	1.493	1.477
4000	2.567	2.567	2.551

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

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 forty separate assays to assess inter-assay precision.

Serum/Plasma Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	458	1371	2545	498	1476	2854
Standard deviation	17.5	82.8	123	38.9	106	256
CV (%)	3.8	6.0	4.8	7.8	7.2	9.0

Cell Culture Supernate Assay

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	141	462	871	156	482	955
Standard deviation	7.53	18.8	37.5	13.5	41.7	96.7
CV (%)	5.3	4.1	4.3	8.7	8.7	10.1

RECOVERY

The recovery of BAFF spiked to levels throughout the range of the assay in various matrices was evaluated. One sample showed < 50% recovery and was not included in the data.

Sample	Average % Recovery	Range
Cell culture media (n=4)	101	87 - 112%
Serum* (n=4)	101	86 - 114%
EDTA plasma* (n=4)	101	85 - 114%
Heparin plasma* (n=4)	98	86 - 110%

*Samples were diluted 2-fold prior to assay as directed in Sample Preparation.

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of BAFF were serially diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum* (n=4)	Heparin plasma* (n=4)	EDTA plasma* (n=4)
1:2	Average % of Expected	100	97	101	100
	Range (%)	95 - 107	92 - 103	100 - 103	100 - 101
1:4	Average % of Expected	98	100	101	98
	Range (%)	94 - 107	94 - 105	98 - 105	91 - 101
1:8	Average % of Expected	102	102	102	104
	Range (%)	94 - 108	97 - 105	101 - 104	102 - 105
1:16	Average % of Expected	100	99	106	104
	Range (%)	95 - 109	96 - 104	105 - 108	100 - 106

*Samples were diluted 2-fold prior to assay as directed in Sample Preparation.

SENSITIVITY

Thirty-four cell culture supernate assays were evaluated and the minimum detectable dose (MDD) ranged from 0.73 - 6.67 pg/mL. The mean MDD was 2.43 pg/mL.

Thirty serum/plasma assays were evaluated and the MDD ranged from 1.50 - 11.9 pg/mL. The mean MDD was 3.38 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human BAFF.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of BAFF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=36)	1169	671 - 2447	283
EDTA plasma (n=36)	1000	609 - 1946	236
Heparin plasma (n=36)	1002	576 - 2132	253

Cell Culture Supernates - Human peripheral blood cells were cultured in DMEM supplemented with 5% fetal calf serum, 50 μ M β -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 μ g/mL PHA. Aliquots of the culture supernate were removed and assayed for levels of natural human BAFF.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	ND	ND
Stimulated	ND	45.9

ND = Non-detectable

Additional cell lines were tested for the presence of human BAFF.

Cell Culture Supernates	Values (pg/mL)
THP-1	100
U937 (7 day culture)	1251
U937 (10 day culture)	18,850

SPECIFICITY

This assay recognizes recombinant and natural human BAFF/BLyS. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human BAFF/BLyS control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

4-1BB Ligand
APRIL
CD27 Ligand
CD30 Ligand
CD40 Ligand
Fas Ligand
GITR Ligand
LIGHT
LT- α 1/ β 2
LT- α 2/ β 1

OX40 Ligand
TNF- α
TNF- β
TRAIL
TRANCE
TWEAK
VEGI

Recombinant mouse:

BCMA
CD27 Ligand
CD30 Ligand
Fas Ligand
LT- α 1/ β 2
LT- α 2/ β 1
OX40 Ligand
TNF- α
TNF- α (truncated)
TRANCE

Recombinant rat:

TNF- α

Recombinant porcine:

TNF- α

Recombinant human BCMA, recombinant human TACI, and recombinant mouse TACI were found to interfere at levels above 1 ng/mL.

Recombinant human BAFF R was found to interfere at levels above 25 ng/mL.

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