

Quantikine™ QuickKit™ ELISA

Human BAFF/BlyS/TNFSF13B Immunoassay

Catalog Number QK0124

SK0124

PK0124

For the quantitative determination of human B Cell Activating Factor Belonging to the TNF Family (BAFF) concentrations in cell culture supernates, serum, plasma, and cell lysates.

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 the 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™ QuickKit™ Human BAFF/BlyS/TNFSF13B Immunoassay is a one-step, 80-minute solid phase ELISA designed to measure human BAFF levels in cell culture supernates, serum, plasma, and cell lysates. It contains recombinant human BAFF and antibodies raised against the recombinant protein. Results obtained for naturally occurring human BAFF showed linear curves that were parallel to the standard curves obtained using the recombinant QuickKit standards. These results indicate that this kit can be used to determine relative mass values for natural human BAFF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled polyclonal capture antibody and an enzyme-linked monoclonal detection antibody, specific for human BAFF. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of BAFF bound. 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the 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™ QuickKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # QK0124	CATALOG # SK0124	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuickKit™ Coated Microplate	899063	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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.*
Human BAFF Standard	899664	2 vials	12 vials	Recombinant human BAFF in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for the reconstitution volume.</i>	Use a freshly reconstituted standard for each assay. Discard after use.
Human BAFF Capture Ab Concentrate	899662	1 vial	6 vials	Lyophilized tagged polyclonal antibody specific for human BAFF.	May be stored for up to 1 month at 2-8 °C.*
Human BAFF Detection Ab Concentrate	899663	1 vial	6 vials	400 µL of a monoclonal antibody specific for human BAFF conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-111	895976	1 vial	6 vials	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-20	895346	1 vial	6 vials	21 mL of a buffered protein base with preservatives. <i>Use diluted 1:2 in this assay.</i>	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
TMB ELISA Substrate	642736	1 vial	6 vials	12 mL of a TMB ELISA substrate	
ELISA Stop Solution	642827	1 vial	6 vials	12 mL of an acid solution	
Plate Sealers	N/A	4 strips	12 strips	Adhesive strips	

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

QK0124 contains sufficient materials to run an ELISA on one 96 well plate.

SK0124 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PK0124). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak has enough reagents to assay 50 microplates (96 wells/plate). Although the inserts are the same as those for the single kit inserts, there are minor differences related to the number of reagents and their container sizes.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
Note: Additional wash buffer is available for purchase ([R&D Systems™, Catalog # WA126](#)).

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
QuickKit™ Coated Microplate	899063	50 plates
Human BAFF Standard	899664	25 vials
Human BAFF Capture Ab Concentrate	899662	50 vials
Human BAFF Detection Ab Concentrate	899663	50 vials
Assay Diluent RD1-111	895976	50 vials
Calibrator Diluent RD5-20	895346	50 vials
Wash Buffer Concentrate	895126	9 bottles
TMB ELISA Substrate	642736	50 vials
ELISA Stop Solution	642827	50 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-technique.com*

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
- Human BAFF Controls (optional; R&D Systems, Catalog # QC335)

For cell lysates samples (optional):

- Lysis Buffer 17 ([R&D Systems, Catalog # 895943](#))

PRECAUTIONS

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.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS 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 and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cells must be lysed prior to assay as directed in the Sample Values section.

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

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$. Avoid repeated freeze-thaw cycles.

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

SAMPLE PREPARATION

Due to matrix effects and endogenous levels, serum, plasma, and cell culture supernate samples require a minimum 2-fold dilution. An example 2-fold dilution is 100 μL sample + 100 μL of Calibrator Diluent RD5-20 (diluted 1:2)*.

For cell lysate samples, quantitation of sample protein concentration using a total protein assay is recommended. The suggested range for total cell lysate protein added is 2-10 $\mu\text{g}/\text{well}$.

Multiple dilutions are recommended for unknown samples.

*Refer to Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human BAFF Capture Antibody Concentrate - Refer to the vial label for reconstitution volume. Reconstitute the Human BAFF Capture Ab Concentrate with Assay Diluent RD1-111. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to rest for a minimum of 5 minutes with gentle agitation before diluting. The 20X capture antibody stock can be stored for up to 4 weeks at 2-8 °C once reconstituted.

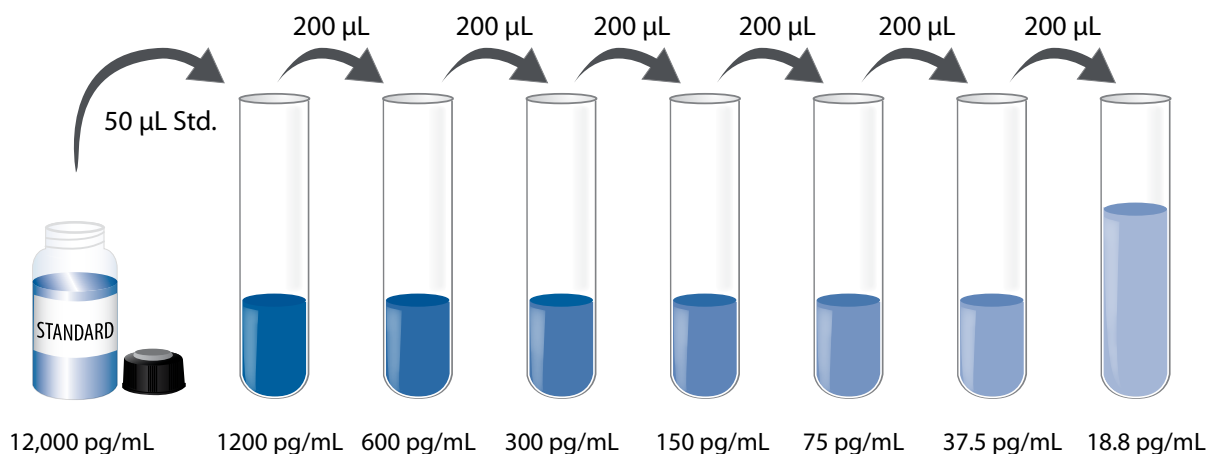
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-111. For a full plate, combine 300 µL of the reconstituted Human BAFF Capture Ab stock and 300 µL of Human BAFF Detection Ab Concentrate with 5.4 mL of Assay Diluent RD1-111 to create 6 mL of Human BAFF Antibody Cocktail.

Calibrator Diluent RD5-20 (diluted 1:2) - Add 10 mL of Calibrator Diluent RD5-20 to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD5-20 (diluted 1:2).

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 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Human BAFF Standard - Refer to vial label for reconstitution volume. Reconstitute the Human BAFF Standard with deionized or distilled water. This reconstitution produces a stock solution of 12,000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions. Use the standard stock within 60 minutes.

Pipette 450 µL of Calibrator Diluent RD5-20 (diluted 1:2) into the 1200 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1200 pg/mL standard serves as the high standard. Calibrator Diluent RD5-20 (diluted 1:2) 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, controls, and samples be assayed in duplicate.

1. Prepare all reagents, samples, and working standards 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 50 μL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 μL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 100 μL of TMB ELISA Substrate solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 100 μL of ELISA 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.
8. 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 require dilution. See Sample Preparation Section.

CALCULATION OF RESULTS

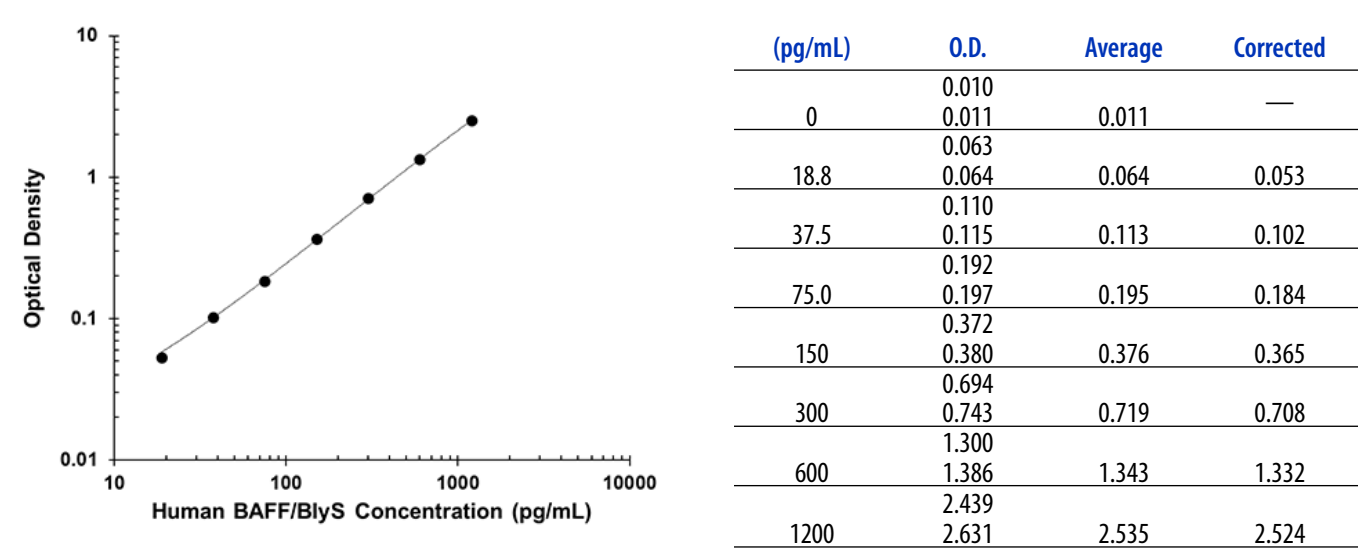
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 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

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



PRECISION

Intra-Assay Precision (Precision within an assay)

Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)

Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least five technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	81.2	605	96.2	558
Standard deviation	2.28	21.9	10.5	34.3
CV (%)	2.8	3.6	10.9	6.2

RECOVERY

The recovery of BAFF is spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range %
Cell culture media (n=2)	97	85-105
Serum (n=2)	95	78-104
EDTA plasma (n=2)	101	85-110
Heparin plasma (n=2)	99	81-108
Lysis Buffer 17 (n=1)	125	117-133

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of BAFF ranged from 0.358-5.23 pg/mL. The mean MDD was 1.80 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.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of BAFF were diluted with Calibrator Diluent RD5-20 (diluted 1:2) to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=2)	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)	Cell lysates (n=1)
1:2	Average % of Expected	97	111	98	104	90
	Range (%)	96-99	109-113	97-99	103-105	n=1
1:4	Average % of Expected	98	114	97	100	89
	Range (%)	98-98	110-118	94-100	98-101	n=1
1:8	Average % of Expected	98	108	90	100	86
	Range (%)	98-98	99-118	86-94	100-100	n=1
1:16	Average % of Expected	99	110	90	100	91
	Range (%)	95-104	99-121	83-98	97-102	n=1

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human BAFF produced at R&D Systems™.

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=10)	634	461-1440	291
EDTA plasma (n=10)	615	416-1360	270
Heparin plasma (n=10)	578	377-1270	256

Cell culture supernates/lysates – U937 cells were seeded at 1×10^5 cells/mL and cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were left untreated for 5 days before harvesting. Cell culture supernates were then centrifuged to remove cells/debris, aliquoted, and assayed for levels of BAFF.

For the cell culture lysates, centrifuged cells were washed with PBS before solubilizing the cells in Lysis Buffer 17 ([Catalog # 895943](#)) with protease inhibitors using 3-5X the cell pellet volume and assayed for levels of human BAFF.

Condition	Cell culture supernates (pg/mL)	Lysates (pg/mL)
Unstimulated, 5 days	4275	447

SPECIFICITY

This assay recognizes natural and recombinant human BAFF.

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 low level recombinant human BAFF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

APRIL

CD40

CD40 Ligand

Lymphotoxin BR

TNF- α

TRANCE

TWEAK

Recombinant human BAFF R interferes at levels > 20 ng/mL in this assay.

Recombinant human BCMA interferes at levels >1 ng/mL in this assay.

Recombinant human TACI interferes at levels > 2 ng/mL in this assay.

Recombinant mouse TACI interferes at levels > 10 ng/mL in this assay.

REFERENCES

1. Lied, G.A. and A. Berstad (2011) Scand. J. Immunol. **73**:1.
2. Liu, Z. and A. Davidson (2011) Trends Immunol. **32**:388.
3. Mackay, F. *et al.* (2010) Immunol. Rev. **237**:205.
4. Moore, P.A. *et al.* (1999) Science **285**:260.
5. Zhang, J. *et al.* (2001) J. Immunol. **166**:6.
6. Cheema, G.S. *et al.* (2001) Arthritis Rheum. **44**:1313.
7. Groom, J. *et al.* (2002) J. Clin. Invest. **109**:59.
8. Mariette, X. *et al.* (2003) Ann. Rheum. Dis. **62**:168.
9. Schneider, P. *et al.* (1999) J. Exp. Med. **189**:1747.
10. Gavin, A.L. *et al.* (2003) J. Biol. Chem. **278**:38220.
11. Nardelli, B. *et al.* (2001) Blood **97**:198.
12. Scapini, P. *et al.* (2003) J. Exp. Med. **197**:297.
13. Kanakaraj, P. *et al.* (2001) Cytokine **13**:25.
14. Roschke, V. *et al.* (2002) J. Immunol. **169**:4314.
15. Gross, J.A. *et al.* (2000) Nature **404**:995.
16. Wu, Y. *et al.* (2000) J. Biol. Chem. **275**:35478.
17. Xia, X.Z. *et al.* (2000) J. Exp. Med. **192**:137.
18. Marsters, S.A. *et al.* (2000) Curr. Biol. **10**:785.
19. Shu, H.B. and H. Johnson (2000) Proc. Natl. Acad. Sci. USA **97**:9156.
20. Yu, G. *et al.* (2000) Nat. Immunol. **1**:252.
21. Thompson, J.S. *et al.* (2001) Science **293**:2108.
22. Madry, C. *et al.* (1998) Int. Immunol. **10**:1693.
23. von Bulow, G.U. and R.J. Bram (1997) Science **278**:138.
24. Gras, M.P. *et al.* (1995) Int. Immunol. **7**:1093.
25. Hatzoglou, A. *et al.* (2000) J. Immunol. **165**:1322.
26. Laabi, Y. *et al.* (1994) Nucleic Acids Res. **22**:1147.
27. Schiemann, B. *et al.* (2001) Science **293**:2111.
28. Gross, J.A. *et al.* (2001) Immunity **15**:289.
29. Mackay, F. *et al.* (1999) J. Exp. Med. **190**:1697.
30. Do, R.K. *et al.* (2000) J. Exp. Med. **192**:953.
31. Batten, M. *et al.* (2000) J. Exp. Med. **192**:1453.
32. Amanna, I.J. *et al.* (2001) J. Immunol. **167**:6069.
33. Amanna, I.J. *et al.* (2003) J. Immunol. **170**:4593.
34. Tardivel, A. *et al.* (2004) Eur. J. Immunol. **34**:509.
35. Khare, S.D. *et al.* (2000) Proc. Natl. Acad. Sci. USA **97**:3370.
36. Tan, S.M. *et al.* (2003) Arthritis Rheum. **48**:982.
37. Kalled, S.L. *et al.* (2003) Expert Opin. Ther. Targets **7**:115.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12								
11								
10								
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3								
2								
1								
	A	B	C	D	E	F	G	H

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