Quantikine® ELISA

Human BAFF/BLyS/TNFSF13B Immunoassay

Catalog Number DBLYS0B
SBLYS0B
PDBLYS0B

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.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

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 δBAFF 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® Human BAFF/BLyS/TNFSF13B Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human BAFF/BLyS in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human BAFF/BLyS and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human 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 human BAFF/BLyS.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human BAFF/BLyS has been pre-coated onto a microplate. Standards 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 human 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 and color develops in proportion to the amount of BAFF/BLyS 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 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DBLYS0B	CATALOG # SBLYS0B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human BAFF/BLyS Microplate	894103	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human BAFF/BLyS Conjugate	894104	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human BAFF/BLyS conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human BAFF/BLyS Standard	894105	1 vial	6 vials	Recombinant human BAFF/BLyS in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-111	895976	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD6Q	895128	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *Use diluted 1:2 in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DBLYS0B contains sufficient materials to run an ELISA on one 96 well plate.
SBLYS0B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDBLYS0B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- 50 mL and 500 mL graduated cylinders.
- 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/BLyS Controls (optional; R&D Systems®, Catalog # QC46).

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.

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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 ≤ -20 °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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly lipemic samples are not suitable for use in this assay.

SAMPLE PREPARATION

Cell culture supernate, serum, and plasma samples require at least a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD6Q (diluted 1:2)

REAGENT PREPARATION

Bring all reagents to room temperature before use.

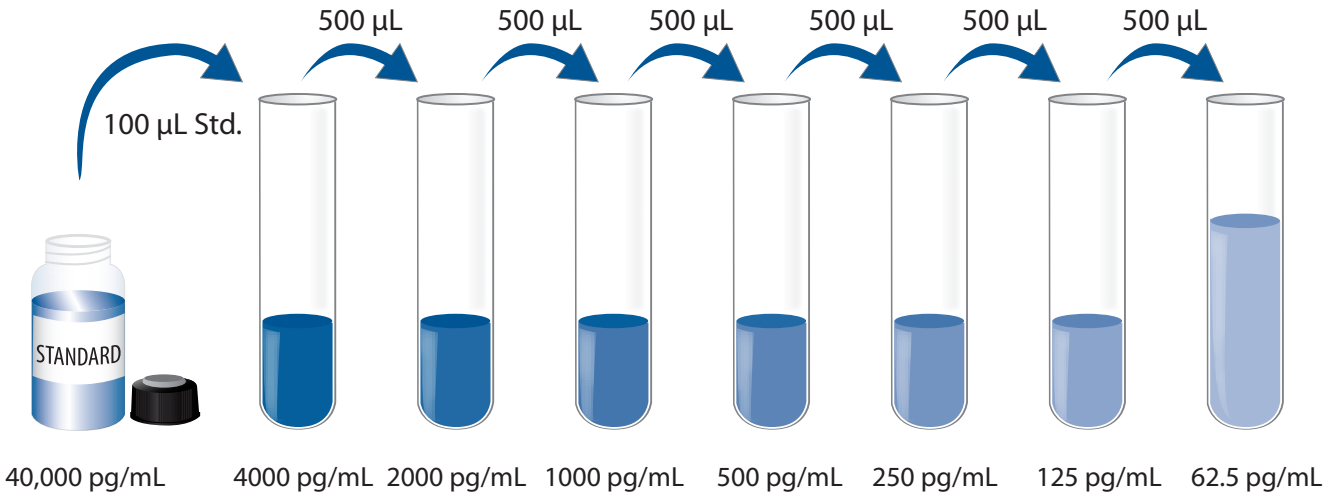
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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

Calibrator Diluent RD6Q (diluted 1:2) - Add 8.0 mL of Calibrator Diluent RD6Q to 8.0 mL of deionized or distilled water to prepare 16 mL of Calibrator Diluent RD6Q (diluted 1:2).

Human BAFF/BLyS Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human BAFF/BLyS Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD6Q (diluted 1:2) into the 4,000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4,000 pg/mL dilution serves as the high standard. Calibrator Diluent RD6Q (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, samples, and controls be assayed in duplicate.

1. Prepare all reagents 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 100 µL of Assay Diluent RD1-111 to each well.
4. Add 50 µL of standard, sample*, or control per well. Cover with the adhesive strip provided. Incubate for 3 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 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 Human BAFF/BLyS Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
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.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

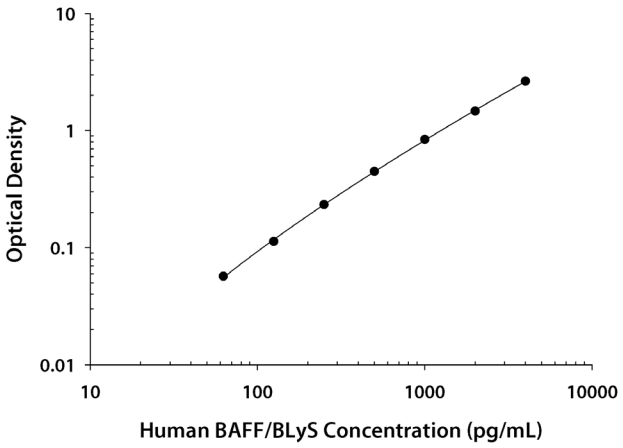
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.)

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 human 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.

Since 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	0.018 0.018	0.018	—
62.5	0.074 0.076	0.075	0.057
125	0.131 0.131	0.131	0.113
250	0.246 0.256	0.251	0.233
500	0.465 0.466	0.466	0.448
1000	0.856 0.862	0.859	0.841
2000	1.471 1.500	1.486	1.468
4000	2.656 2.656	2.656	2.638

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 twenty 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	20	20	20
Mean (pg/mL)	433	1000	2343	474	1118	2111
Standard deviation	28.2	72.0	78.6	46.8	112	244
CV (%)	6.5	7.2	3.4	9.9	10.0	11.6

RECOVERY

The recovery of human BAFF/BLyS spiked to three different levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to this assay.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	90-103%
Serum (n=4)	93	84-106%
EDTA plasma (n=4)	94	84-101%
Heparin plasma (n=4)	94	89-107%

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of human BAFF/BLyS ranged from 1.01-6.44 pg/mL. The mean MDD was 2.68 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 and/or spiked with high concentrations of human BAFF/BLyS were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	101	103	104	103
	Range (%)	97-104	101-106	99-112	100-106
1:4	Average % of Expected	98	101	104	104
	Range (%)	95-102	97-107	98-113	99-111
1:8	Average % of Expected	95	101	105	104
	Range (%)	92-98	97-108	99-115	99-112
1:16	Average % of Expected	95	107	111	111
	Range (%)	92-99	102-111	106-118	106-118

*Samples were diluted prior to assay.

CALIBRATION

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

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human BAFF/BLyS 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)	850	584-1186	155
EDTA plasma (n=36)	790	501-1078	147
Heparin plasma (n=36)	765	528-1073	143

Cell Culture Supernates - U937 human histiocytic lymphoma cells were cultured in RPMI supplemented with 10% fetal bovine serum and 2 mM L-glutamine, and incubated for 7 days at 37 °C. An aliquot of the cell culture supernate was removed, assayed for human BAFF/BLyS, and measured 3082 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant 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
BAFF R
BCMA
CD27 Ligand
CD30 Ligand
CD40 Ligand
Fas Ligand
GITR Ligand
LIGHT
LT-α1/β2
LT-α2/β1
OX40 Ligand
TNF-α
TNF-β
TRAIL
TRANCE
TWEAK
VEGI

Recombinant mouse:

BAFF R
BCMA
CD27 Ligand
CD30 Ligand
Fas Ligand
LT-α1/β2
LT-α2/β1
OX40 Ligand
TACI
TNF-α
TNF-α (truncated)
TRANCE

Recombinant rat:

TNF-α

Recombinant porcine:

TNF-α

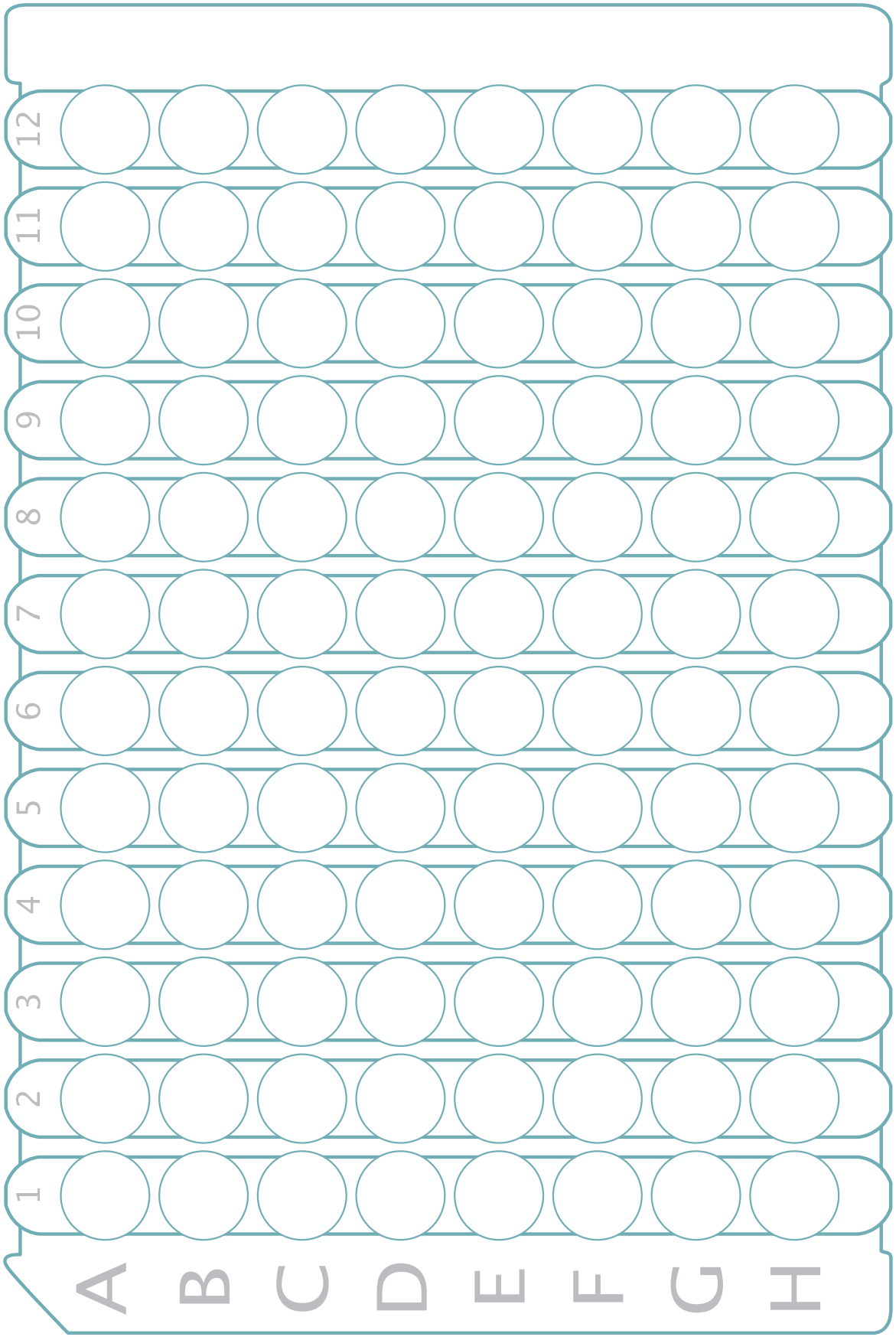
Recombinant human TACI interferes at levels > 3.13 ng/mL.

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.



NOTES

NOTES