

Quantikine[™] ELISA

Human BCMA/TNFRSF17 Immunoassay

Catalog Number DBCMA0
SBCMA0
PDBCMA0

For the quantitative determination of human B Cell Maturation Antigen (BCMA) concentrations in cell culture supernates, serum, plasma, saliva, and urine.

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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Manufactured and Distributed by:

USA R&D Systems, Inc.

614 McKinley Place NE, Minneapolis, MN 55413

TEL: 800 343 7475 612 379 2956

FAX: 612 656 4400

E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.

19 Barton Lane, Abingdon Science Park

Abingdon OX14 3NB, UK

TEL: +44 (0)1235 529449

FAX: +44 (0)1235 533420

E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.

Unit 1901, Tower 3, Raffles City Changning Office,

1193 Changning Road, Shanghai PRC 200051

TEL: +86 (21) 52380373 (400) 821-3475

FAX: +86 (21) 52371001

E-MAIL: info.cn@bio-techne.com

INTRODUCTION

B Cell Maturation Antigen (BCMA) is a transmembrane protein that holds significant importance in the field of immunology and oncology. It is primarily expressed on the surface of plasma cells, the terminally differentiated B cells responsible for producing antibodies. BCMA plays a pivotal role in regulating the survival, proliferation, and maintenance of plasma cells, making it a critical target in therapeutic research for various B cell-related diseases, notably multiple myeloma.

BCMA belongs to the tumor necrosis factor receptor superfamily (TNFRSF), which encompasses a collection of cell surface receptors governing various cellular processes, including survival and differentiation. Interactions between BCMA and its ligands, A Proliferation-Inducing Ligand (APRIL) and B Cell Activating Factor (BAFF), trigger intricate signaling pathways that are pivotal for plasma cell survival and antibody generation (1-2). This interaction activates NF- κ B, elk-1, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase, further underlining its significance.

The relevance of BCMA extends beyond its role in plasma cell biology. Research has shown that Hodgkin lymphoma cells also express TACI and BCMA receptors, responding to BAFF and APRIL with survival and proliferation signals (3). The involvement of BCMA in protecting myeloma cells from apoptosis induced by IL-6 deprivation and dexamethasone underscores its intricate connections with disease progression (4).

ELISAs represent a widely used analytical technique for detecting and quantifying specific proteins in various samples. In the context of ELISAs, BCMA can serve as a target antigen, enabling the quantification of its presence in different samples. This approach has proven valuable in elucidating the role of BCMA in various physiological and pathological contexts.

The Quantikine™ Human B Cell Maturation Antigen (BCMA) Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human BCMA in cell culture supernates, serum, plasma, saliva, and urine. It contains recombinant human BCMA and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human BCMA 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 BCMA.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human BCMA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BCMA present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human BCMA 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 BCMA 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 # DBCMA0	CATALOG # SBCMA0	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human BCMA Microplate	899535	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human BCMA.	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 BCMA Standard	899537	2 vials	6 vials	Recombinant human BCMA in a buffered protein base with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Use a new standard for each assay. Discard after use.
Human BCMA Conjugate	899536	1 vial	6 vials	21 mL of a monoclonal antibody specific for human BCMA conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-98	895596	1 vial	6 vials	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26	895525	1 vial	6 vials	21 mL of a concentrated 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>	
Color Reagent A	895000	1 vial	6 vials	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL of 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	4 adhesive strips.	

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

DBCMA0 contains sufficient materials to run an ELISA on one 96 well plate.

SBCMA0 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDBCMA0). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- 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®, # WA126).

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human BCMA Microplate	899535	50 plates
Human BCMA Standard*	899537	25 vials
Human BCMA Conjugate	899536	50 vials
Assay Diluent RD1-98	895596	50 vials
Calibrator Diluent RD5-26	895525	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.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 BCMA Controls (optional; R&D Systems, Catalog # QC308)

PRECAUTIONS

BCMA is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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

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

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Due to high endogenous levels, serum and plasma samples require a 20-fold dilution prior to assay. A suggested 20-fold dilution is 10 μ L of serum + 190 μ L of Calibrator Diluent RD5-26 (diluted 1:2)*.

Multiple dilutions are recommended for unknown samples.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: BCMA is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

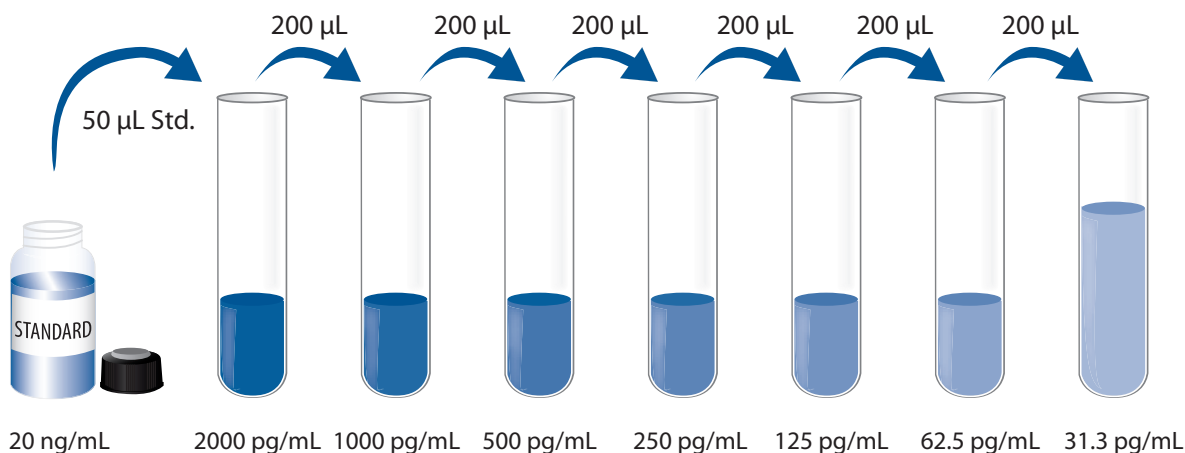
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5-26 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD5-26 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-26 (diluted 1:2).

Human BCMA Standard - Refer to the vial label for reconstitution volume. Reconstitute the Human BCMA Standard with deionized or distilled water. This reconstitution produces a stock solution of 20 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Pipette 450 μ L of Calibrator Diluent RD5-26 (diluted 1:2) into the 2000 pg/mL tube. Pipette 200 μ L 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 RD5-26 (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.

Note: *BCMA is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 μ L of Assay Diluent RD1-98 to each well.
4. Add 50 μ L 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 three times for a total of four washes. Wash by filling each well with Wash Buffer (400 μ L) 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 μ L of Human BCMA 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 200 μ L of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 μ L 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 may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

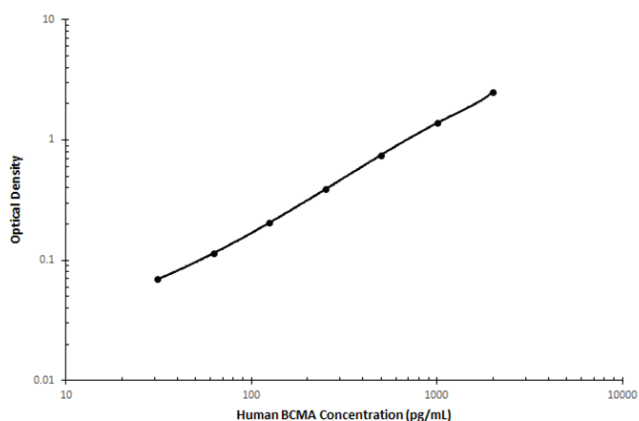
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 BCMA 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 prior to assay, 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	0.020 0.022	0.021	—
31.3	0.069 0.070	0.070	0.049
62.5	0.112 0.116	0.114	0.093
125	0.199 0.208	0.204	0.183
250	0.385 0.393	0.389	0.368
500	0.750 0.750	0.750	0.729
1000	1.380 1.380	1.380	1.359
2000	2.480 2.521	2.501	2.480

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.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	210	700	1308	211	659	1314
Standard deviation	9.49	22.2	43.6	10.2	48.1	73.4
CV (%)	4.5	3.2	3.3	4.8	7.3	5.6

RECOVERY

The recovery of human BCMA spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Media (n=4)	99	80-106%
Saliva (n=4)	96	83-115%
Urine (n=4)	102	94-112%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human BCMA were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

Samples		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	97	102	100	101	106	96
	Range (%)	94-100	98-107	97-104	96-107	103-108	94-99
1:4	Average % of Expected	92	101	101	98	106	94
	Range (%)	89-96	97-104	100-102	92-100	99-111	90-97
1:8	Average % of Expected	89	101	99	100	106	92
	Range (%)	86-93	96-108	97-102	95-103	98-111	89-96
1:16	Average % of Expected	89	101	97	103	100	83
	Range (%)	85-94	89-110	90-106	88-112	86-116	78-89

SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of human BCMA ranged from 3.44-10.8 pg/mL. The mean MDD was 6.46 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 recombinant human BCMA manufactured at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human BCMA 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=30)	15,561	6976-23,239	5054
EDTA plasma (n=30)	15,162	7331-24,489	4913
Heparin plasma (n=30)	15,892	8036-24,919	5269

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Saliva (n=10)	99.1	70	ND-240
Urine (n=10)	207	80	ND-456

ND=Non-detectable

Cell Culture Supernates:

Human B cells were isolated from PBMCs via the MagCelect™ Human B Cell Isolation Kit ([Catalog # MAGH103](#)). B cells were seeded at approximately 5×10^6 /mL in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and then left untreated or expanded with the CellXVivo™ Human B Cell Expansion Kit ([Catalog # CDK005](#)) for 5 days prior to conditioned media harvest. An aliquot of the cell culture supernate was removed, assayed for human BCMA, and measured.

Condition	Day 5 (pg/mL)
Unstimulated	ND
Stimulated	3344

ND=Non-Detectable

SPECIFICITY

This assay recognizes natural and recombinant human BCMA.

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 BCMA control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

BAFF
BAFF R
TACI
TRAF-2
TRAF-3
TRAF-5
TRAF-6

Other recombinants:

cyno macaque BCMA

Recombinant mouse BCMA does not interfere but does cross-react approximately 0.24 % in this assay.

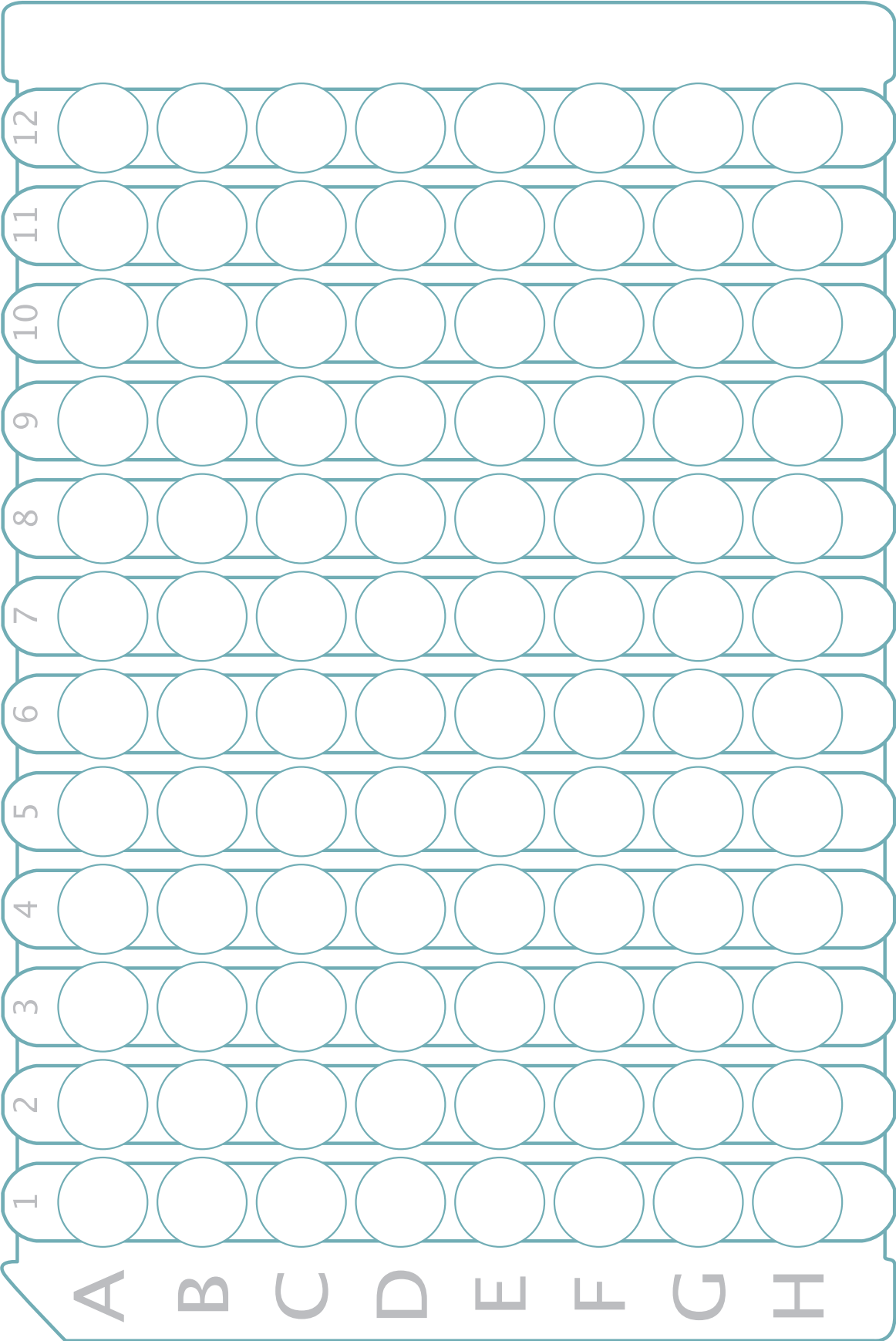
Recombinant human APRIL does not cross-react but does interfere at concentrations greater than 2000 pg/mL.

REFERENCES

1. Hatzoglou, A. *et al.* (2000) *Int Immunol.* **165**:1322
2. Madry, C. *et al.* (1998) *Int Immunol.* **10**:1693
3. Chiu, A. *et al.* (2007) *Blood.* **109**:729
4. Moreaux, J. *et al.* (2004) *Blood.* **103**:3148

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES

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