bio-techne®

R&D SYSTEMS

Quantikine™ ELISA

Mouse ST2/IL-33R Immunoassay

Catalog Number MST200

For the quantitative determination of mouse ST2/IL-33R 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

SECTION PAGE

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

ST2, also known as IL-33 R, Fit-1, and T1, is an Interleukin-1 receptor family glycoprotein that contributes to Th2 immune responses (1, 2). ST2 is expressed on the surface of mast cells, activated Th2 cells, macrophages, and cardiac myocytes (3-8). Mouse ST2 consists of a 306 amino acid (aa) extracellular domain (ECD) with three Ig-like domains, a 23 aa transmembrane segment, and a 212 aa cytoplasmic domain with an intracellular TIR domain (9, 10). A soluble 60 kDa isoform of mouse ST2 is generated by alternate promoter usage (11). Within the ECD, mouse ST2 shares 68% and 81% aa sequence identity with human and rat ST2, respectively.

ST2 binds IL-33, a pro-inflammatory IL-1 family cytokine with intracellular and extracellular activities. IL-33 is constitutively expressed in smooth muscle and airway epithelia (3). It is upregulated by inflammatory stimulation in these cells, keratinocytes, dermal fibroblasts, and by mechanical strain in cardiac fibroblasts (3, 12). Similar to IL-1, the N-terminal propeptide of IL-33 is cleaved intracellularly to release the C-terminal fragment which is exported as the active cytokine (3, 13). IL-33 binding induces the association of transmembrane ST2 with IL-1 RAcP, a shared signaling subunit that also associates with IL-1 RI and IL-1 Rrp2/IL-1 R6 (14, 15). Soluble ST2 also binds IL-33 and functions as a decoy receptor that blocks the ability of IL-33 to signal through transmembrane ST2 (12, 14, 16-18).

Secreted IL-33 promotes Th2-biased immune responses, resulting in eosinophilia and allergic inflammation (19). It induces the upregulation of inflammatory cytokines and chemokines in Th2 cells and mast cells (3, 20, 21). It also functions as a chemoattractant for Th2 cells to sites of inflammation (22). In addition to its role in promoting mast cell and Th2 dependent inflammation, transmembrane ST2 activation enhances inflammation-associated hypernociception and protects from atherosclerosis and cardiac myocyte hypertrophy (12, 16, 17). The soluble ST2 isoform is elevated in the serum under inflammatory conditions including allergic asthma, sepsis, trauma, dengue fever, pulmonary disease, and lupus (18, 23-27). Serum ST2 elevation is also associated with multiple aspects of heart failure including aortic stenosis, congestive cardiomyopathy, and risk of cardiovascular heart failure and death (28-33).

The Quantikine™ Mouse ST2/IL-33R Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse ST2 in cell culture supernates, serum, and plasma. It contains *Sf*21-expressed recombinant mouse ST2 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural mouse ST2 showed dose-response 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 mouse ST2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse ST2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any ST2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse ST2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of ST2 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

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

TECHNICAL HINTS

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

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse ST2 Microplate	893902	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse ST2.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse ST2 Standard	893904	Recombinant mouse ST2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C for up to 1 month.* Avoid repeated freeze-thaw cycles.
Mouse ST2 Control	893905	Recombinant mouse ST2 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse ST2 Conjugate	893903	12 mL of a monoclonal antibody specific for mouse ST2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-41	895514	12 mL of a concentrated buffered protein base with preservatives.	
Calibrator Diluent RD5-24	895325	21 mL of a buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

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

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.

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

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

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

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

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-24 (diluted 1:5)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse ST2 Control - Reconstitute the control with 1 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

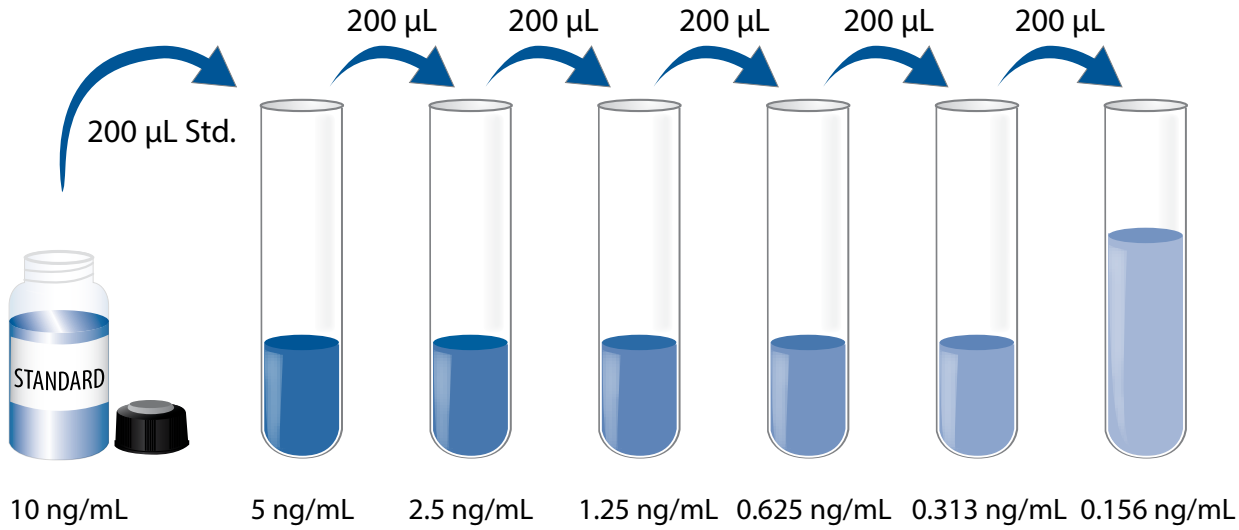
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-24 (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5-24 to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5-24 (diluted 1:5).

Mouse ST2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse ST2 Standard with Calibrator Diluent RD5-24 (diluted 1:5). This reconstitution produces a stock solution of 10 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-24 (diluted 1:5) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse ST2 Standard (10 ng/mL) serves as the high standard. Calibrator Diluent RD5-24 (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

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

1. Prepare all reagents, working standards, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-41 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse ST2 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

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

CALCULATION OF RESULTS

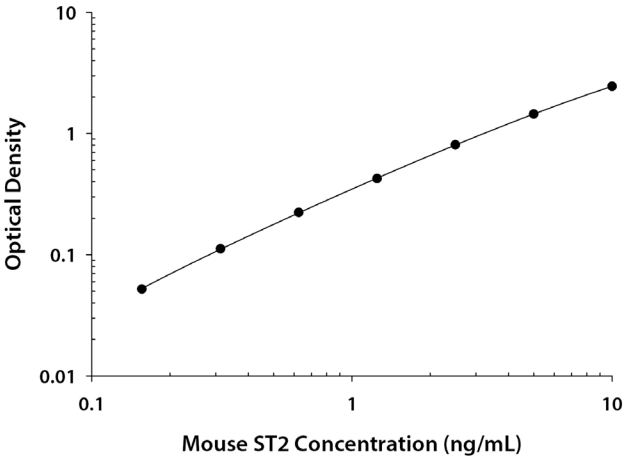
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse ST2 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.



(ng/mL)	O.D.	Average	Corrected
0	0.011 0.012	0.012	—
0.156	0.063 0.065	0.064	0.052
0.313	0.121 0.127	0.124	0.112
0.625	0.224 0.245	0.235	0.223
1.25	0.424 0.450	0.437	0.425
2.5	0.810 0.827	0.819	0.807
5	1.485 1.493	1.489	1.447
10	2.460 2.466	2.463	2.451

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. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.465	1.34	3.81	0.512	1.43	3.94
Standard deviation	0.019	0.042	0.106	0.047	0.082	0.138
CV (%)	4.1	3.1	2.8	9.2	5.7	3.5

RECOVERY

The recovery of mouse ST2 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	98	81-111%
Serum* (n=4)	103	89-113%
EDTA plasma* (n=4)	104	92-118%
Heparin plasma* (n=4)	94	86-103%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse ST2 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	94	99	96	98
	Range (%)	87-98	95-104	91-99	94-101
1:4	Average % of Expected	91	99	95	98
	Range (%)	82-96	93-105	91-98	91-101
1:8	Average % of Expected	90	100	94	97
	Range (%)	84-95	92-108	90-100	91-104
1:16	Average % of Expected	90	101	95	95
	Range (%)	84-98	93-112	84-105	88-101

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Fifty-three assays were evaluated and the minimum detectable dose (MDD) of mouse ST2 ranged from 0.004-0.059 ng/mL. The mean MDD was 0.014 ng/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 *Sf*21-expressed recombinant mouse ST2/IL-33 R produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse ST2 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	41.5	27.3-64.7	8.81
EDTA plasma (n=20)	38.8	28.6-58.3	8.68
Heparin plasma (n=20)	38.6	27.6-50.7	6.59

Cell Culture Supernates:
Liver organ tissue from one mouse was homogenized and seeded in 100 mL of RPMI 1640 supplemented with 10% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate for 3 days. An aliquot of the cell culture supernate was removed, assayed for mouse ST2, and measured 0.165 ng/mL.

NIH-3T3 mouse embryonic fibroblast cells were seeded at 0.3 x 10^5 cells/flask and cultured in DMEM supplemented with 10% fetal bovine serum for 3-4 days. An aliquot of the cell culture supernate was removed, assayed for mouse ST2, and measured 1632 ng/mL.

Mouse Embryonic Fibroblast (MEF) Conditioned Media (R&D Systems®, # AR005) was prepared according to the protocol published by Xu, C. *et al.* (7). Serum-free media (80% Knockout™ DMEM, 20% Knockout serum replacement, 1% MEM non-essential amino acids, 2 mM GlutaMAX™, 0.1 mM β-mercaptoethanol, 4 ng/mL FGF basic) was conditioned by γ-irradiated CF-1 fibroblasts at 37 °C for 24 hours. An aliquot of the cell culture supernate was removed, assayed for mouse ST2, and measured 162 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse ST2.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 100 ng/mL in a mid-range mouse ST2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-1α
IL-1β
IL-1ra
IL-1 R1
IL-1 R2
IL-1 RAPL2/IL-1 R9
IL-1 Rrp2/IL-1 R6
IL-18
IL-33 (aa 1-108)
SIGIRR
TLR1
TLR2
TLR3
TLR4
TLR5
TLR6
TLR7
TLR8
TLR12

Recombinant human:

IL-1 RAcp/IL-1 R3
IL-33 (aa 1-111)
IL-33 (aa 112-270)
ST2/IL-33 R
TIGIRR

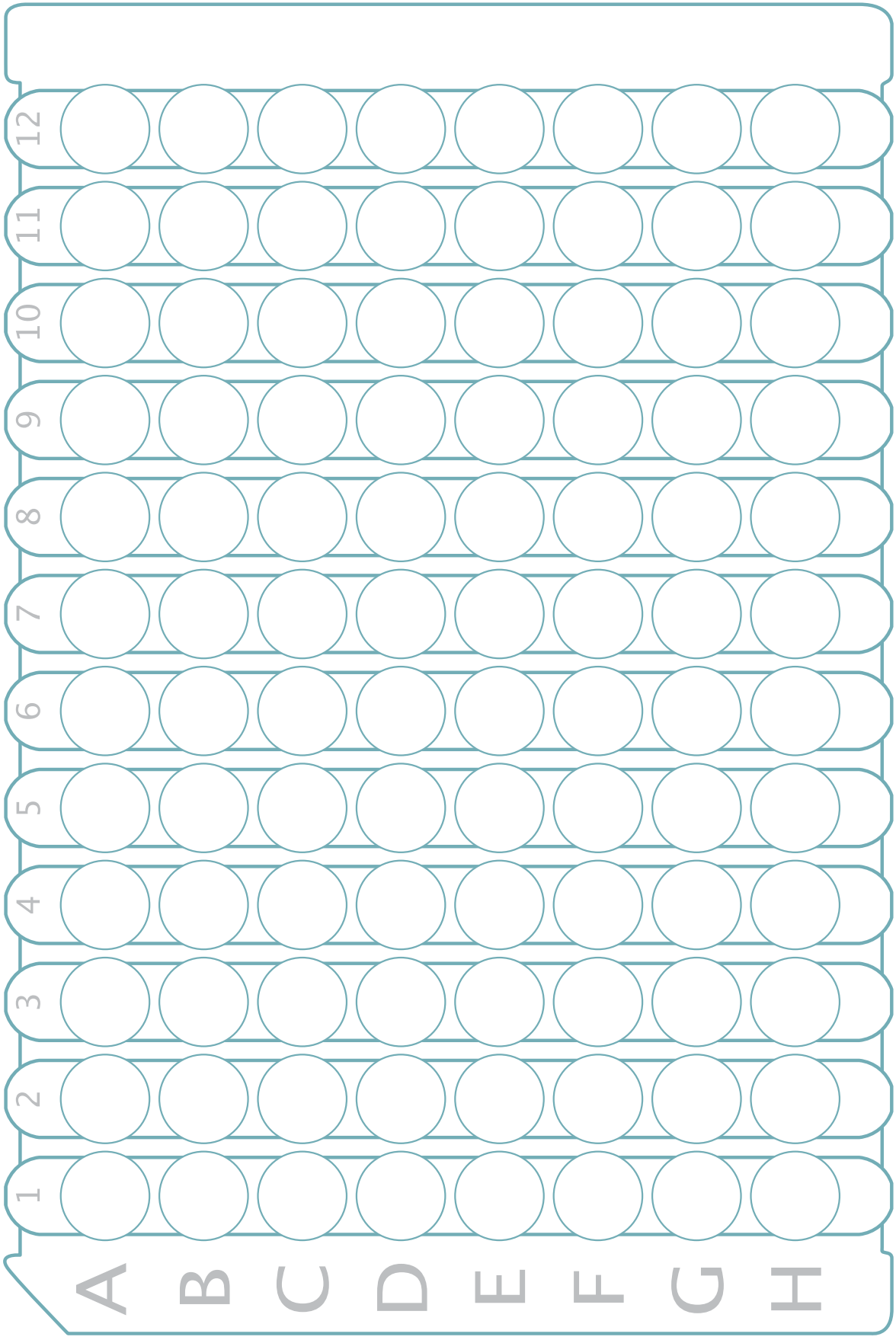
Recombinant mouse IL-33 (aa 109-266) does not cross-react in this assay, but it does interfere at concentrations > 0.188 ng/mL.

REFERENCES

1. Kakkar, R. and R.T. Lee (2008) Nat. Rev. Drug Discov. **7**:827.
2. Barksby, H.E. *et al.* (2007) Clin. Exp. Immunol. **149**:217.
3. Schmitz, J. *et al.* (2005) Immunity **23**:479.
4. Lecart, S. *et al.* (2002) Eur. J. Immunol. **32**:2979.
5. Brint, E.K. *et al.* (2004) Nat. Immunol. **5**:373.
6. Weinberg, E.O. *et al.* (2002) Circulation **106**:2961.
7. Xu, D. *et al.* (1998) J. Exp. Med. **187**:787.
8. Lohning, M. *et al.* (1998) Proc. Natl. Acad. Sci. USA **95**:6930.
9. Klemenz, R. *et al.* (1989) Proc. Natl. Acad. Sci. USA **86**:5708.
10. Tominaga, S. *et al.* (1991) Biochim. Biophys. Acta **1090**:1.
11. Bergers, G. *et al.* (1994) EMBO J. **13**:1176.
12. Sanada, S. *et al.* (2007) J. Clin. Invest. **117**:1538.
13. Black, R.A. *et al.* (1989) J. Biol. Chem. **264**:5323.
14. Palmer, G. *et al.* (2008) Cytokine **42**:358.
15. Chackerian, A.A. *et al.* (2007) J. Immunol. **179**:2551.
16. Verri Jr., W.A. *et al.* (2008) Proc. Natl. Acad. Sci. USA **105**:2723.
17. Miller, A.M. *et al.* (2008) J. Exp. Med. **205**:339.
18. Hayakawa, H. *et al.* (2007) J. Biol. Chem. **282**:26369.
19. Kato, A. and R.P. Schleimer (2007) Curr. Opin. Immunol. **19**:711.
20. Allakhverdi, Z. *et al.* (2007) J. Immunol. **179**:2051.
21. Moulin, D. *et al.* (2007) Cytokine **40**:216.
22. Komai-Koma, M. *et al.* (2007) Eur. J. Immunol. **37**:2779.
23. Martinez-Rumayor, A. *et al.* (2008) Am. J. Clin. Pathol. **130**:578.
24. Becerra, A. *et al.* (2008) Cytokine **41**:114.
25. Brunner, M. *et al.* (2004) Intensive Care Med. **30**:1468.
26. Tajima, S. *et al.* (2003) Chest **124**:1206.
27. Mok, M.Y. *et al.* (2010) Rheumatology **49**:520.
28. Bartunek, J. *et al.* (2008) J. Am. Coll. Cardiol. **52**:2166.
29. Szerafin, T. *et al.* (2009) Thorac. Cardiovasc. Surg. **57**:25.
30. Sabatine, M.S. *et al.* (2008) Circulation **117**:1936.
31. Mueller, T. *et al.* (2008) Clin. Chem. **54**:752.
32. Shimpo, M. *et al.* (2004) Circulation **109**:2186.
33. Weinberg, E.O. *et al.* (2003) Circulation **107**:721.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES