Quantikine® ELISA

Mouse/Rat TREM-1 Immunoassay

Catalog Number MTRM10

For the quantitative determination of mouse or rat Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) concentrations in cell culture supernates, tissue homogenates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	2
TECHNICAL HINTS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
PRECAUTIONS	4
SAMPLE COLLECTION & STORAGE	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
SENSITIVITY	8
LINEARITY	9
CALIBRATION	9
SAMPLE VALUES	10
SPECIFICITY	11
REFERENCES	11
PLATE LAYOUT	12

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

The TREM (Triggering Teceptor Expressed on Myeloid cells) gene cluster (at mouse chromosome 17 and human chromosome 6) encodes both activating and inhibitory type I transmembrane single immunoglobulin variable (IgV) domain receptors that are expressed on cells of the innate immune system (1-6). These activating and inhibitory receptors are named TREMs and TREM-like transcripts (TLTs), respectively. TREMs have a charged lysine residue in their transmembrane domain that allows for interaction with an ITAM (immunoreceptor tyrosine-based activation motif)-containing transmembrane adapter protein DAP12, which triggers cellular activation (4, 7, 8). In contrast, TLTs lack a transmembrane charged lysine residue, but have a long cytoplasmic domain with one or more immunoreceptor tyrosine-based inhibitory motif (ITIM) that transduces cellular inhibition signals (4, 6).

Mouse TREM-1 is a 30 kDa monomeric transmembrane activating receptor that is synthesized as a 230 amino acid (aa) residue precursor with a 20 aa signal peptide, a 188 aa extracellular domain, a 23 aa transmembrane domain, and a short 7 aa cytoplasmic domain (4, 8, 9). The amino acid sequence of the extracellular domain of mouse TREM-1 shares 80% and 41% identity with that of human (10) and rat (11) TREM-1, respectively. TREM-1 is expressed by neutrophils, monocytes, various macrophage subsets, and Kupffer cells (12, 13). During infection by Gram-positive and Gram-negative bacteria and fungi, the expression of membrane-bound TREM-1 is highly upregulated (4, 14, 15). A soluble form of TREM-1 is also released from the cells and can be measured in biological fluids (14, 15). Since a mouse mRNA alternative splice variant predicted to encode a soluble mouse TREM-1 isoform has not been described, mouse soluble TREM-1 likely arises by proteolytic processing of the membrane-bound form (17).

TREM-1 is an important regulator in innate immunity and functions to amplify inflammation in response to infection (16, 18). A natural ligand for TREM-1 has not been identified. Activation of TREM-1 on monocytes by agonist antibodies in the presence of TLR (Toll-like receptor) ligands synergistically increases the production of pro-inflammatory chemokines and cytokines, and decreases the production of anti-inflammatory IL-10 (16). Monocyte TREM-1 activation also induces the differentiation of primary monocytes into immature dendritic cells. On neutrophils, TREM-1 ligation induces degranulation, respiratory burst and phagocytosis synergistically with TLR ligands (18). In contrast, it antagonizes the TLR-mediated anti-apoptotic effects on neutrophils.

The Quantikine Mouse/Rat TREM-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse or rat TREM-1 in cell culture supernates, tissue homogenates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse TREM-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural TREM-1 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 naturally occurring mouse and rat TREM-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse/rat TREM-1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any TREM-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse/rat TREM-1 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 TREM-1 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, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- 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/Rat TREM-1 Microplate	893013	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse/rat TREM-1.	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/Rat TREM-1 Conjugate	893014	12 mL of a polyclonal antibody specific for mouse/rat TREM-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse/Rat TREM-1 Standard	893015	Recombinant mouse TREM-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse/Rat TREM-1 Control	893016	Recombinant mouse TREM-1 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Assay Diluent RD1-41	895514	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-16	895302	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of a 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.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.

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

Tissue Homogenates - Mouse lung and liver from eight mice and rat heart and kidney from one rat were rinsed with PBS to remove excess blood, homogenized in 20 mL of PBS (for mouse tissue) or 5-10 mL of PBS (for rat tissue) and stored overnight at ≤ -20 °C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Assay the supernate 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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

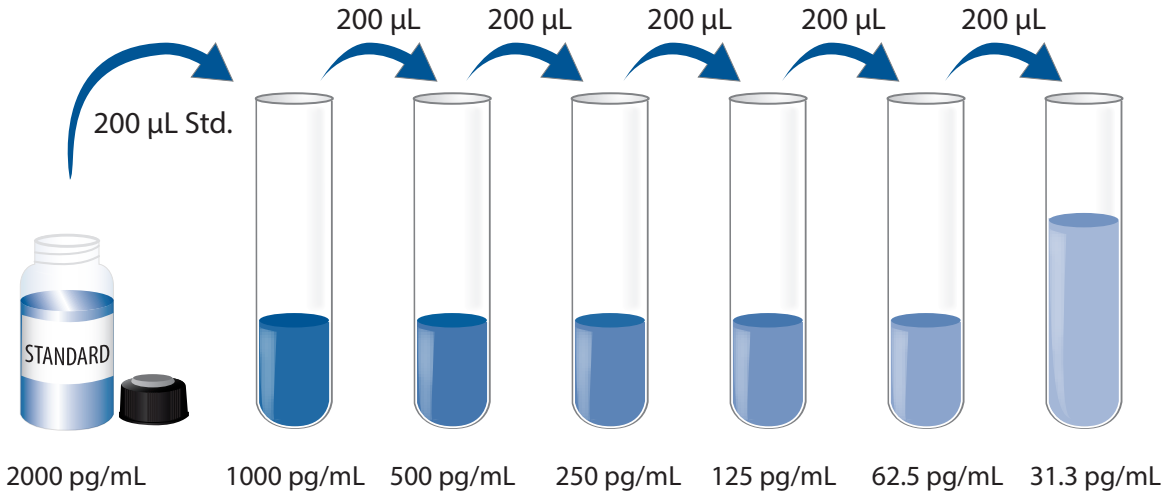
Mouse/Rat TREM-1 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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 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.

Mouse/Rat TREM-1 Standard - Refer to the vial label for reconstitution volume. Reconstitute the Mouse/Rat TREM-1 Standard with Calibrator Diluent RD5-16. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/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 RD5-16 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse/Rat TREM-1 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-16 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, Control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, 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/Rat TREM-1 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.

For research use only. Not for use in diagnostic procedures.

CALCULATION OF RESULTS

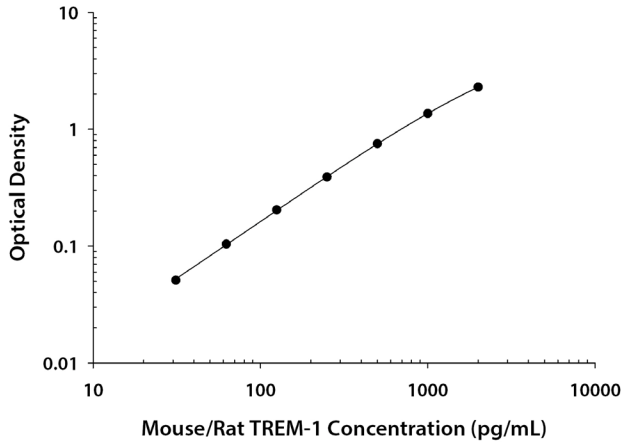
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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/rat TREM-1 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.



(pg/mL)	O.D.	Average	Corrected
0	0.068 0.070	0.069	—
31.3	0.118 0.121	0.120	0.051
62.5	0.172 0.173	0.173	0.104
125	0.272 0.274	0.273	0.204
250	0.459 0.459	0.459	0.390
500	0.807 0.832	0.820	0.751
1000	1.416 1.445	1.431	1.362
2000	2.323 2.399	2.361	2.292

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-six 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	26	26	26
Mean (pg/mL)	79.8	248	1222	79.3	227	1160
Standard deviation	4.5	22.0	51.6	6.3	14.0	62.2
CV (%)	5.6	8.9	4.2	7.9	6.2	5.4

RECOVERY

The recovery of mouse/rat TREM-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Mouse Samples	Average % Recovery	Range
Cell culture supernates (n=5)	98	87-109%
Tissue homogenates (n=2)	100	91-104%
Serum (n=5)	98	81-106%
EDTA plasma (n=6)	99	90-108%
Heparin plasma (n=5)	101	89-110%

Rat Samples	Average % Recovery	Range
Cell culture supernates (n=5)	97	90-109%
Tissue homogenates (n=2)	99	95-103%
Serum (n=4)	107	84-120%
EDTA plasma (n=5)	100	85-112%
Heparin plasma (n=5)	99	85-113%

SENSITIVITY

Sixteen assays were evaluated and the minimum detectable dose (MDD) of mouse/rat TREM-1 ranged from 2.3-5.4 pg/mL. The mean MDD was 3.7 pg/mL.

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

For research use only. Not for use in diagnostic procedures.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse/rat TREM-1 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

Mouse Samples		Cell culture supernates (n=5)	Tissue homogenates (n=2)	Serum (n=7)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	98	95	100	98	97
	Range (%)	91-103	93-97	97-102	94-101	93-105
1:4	Average % of Expected	97	94	98	96	94
	Range (%)	90-101	91-96	93-105	92-102	88-104
1:8	Average % of Expected	96	93	99	96	96
	Range (%)	90-101	92-94	91-114	92-104	91-105
1:16	Average % of Expected	96	94	97	94	96
	Range (%)	90-101	94-95	90-112	88-99	91-99

Rat Samples		Cell culture supernates (n=6)	Tissue homogenates (n=2)	Serum (n=4)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	100	96	101	100	98
	Range (%)	94-103	96-96	98-103	95-106	96-100
1:4	Average % of Expected	99	93	103	98	95
	Range (%)	93-107	90-97	100-107	92-104	94-96
1:8	Average % of Expected	99	95	110	98	94
	Range (%)	91-108	93-97	109-112	91-105	92-98
1:16	Average % of Expected	101	93	110	97	89
	Range (%)	90-108	90-96	108-113	83-104	87-91

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse TREM-1/Fc Chimera produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse/rat TREM-1 in this assay.

Mouse Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=21)	70.0	39-139	24.5
EDTA plasma (n=21)	63.8	31-164	33.5
Heparin plasma (n=22)	73.8	40-107	17.5

Rat Samples	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	746	550-958	126
EDTA plasma (n=21)	896	183-1738	478
Heparin plasma (n=21)	585	356-773	113

Cell Culture Supernates:
J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM containing 10% fetal bovine serum and stimulated with 2.5 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed fornouse/rat TREM-1, and measured 132 pg/mL.

Rat lung condtioned media (1 lung, 1-2 mm pieces) was cultured for 3 days in 25 mL of RPMI supplemented with 10% fetal bovine serum and stimulated with 10 µg/mL Con A. An aliquot of the cell culture supernate was removed, assayed for mouse/rat TREM-1, and measured 805 pg/mL.

Tissue Homogenates:
Mouse tissue homogenates from mouse lung and liver tissue were removed, assayed for mouse/rat TREM-1, and measured 76 and 61 pg/mL, respectively.

Rat tissue homogenates from rat heart and kidney tissue were removed, assayed for mouse/rat TREM-1, and measured 70 and 104 pg/mL, respectively.

For research use only. Not for use in diagnostic procedures.

SPECIFICITY

This assay recognizes natural and recombinant mouse and rat TREM-1.

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 mouse/rat TREM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IFN-γ
IL-1α
IL-1β
IL-2
IL-6
JE/MCP-1
TLT-1/TREML1
TNF-α
TREM-2b
TREM-3

Recombinant human:

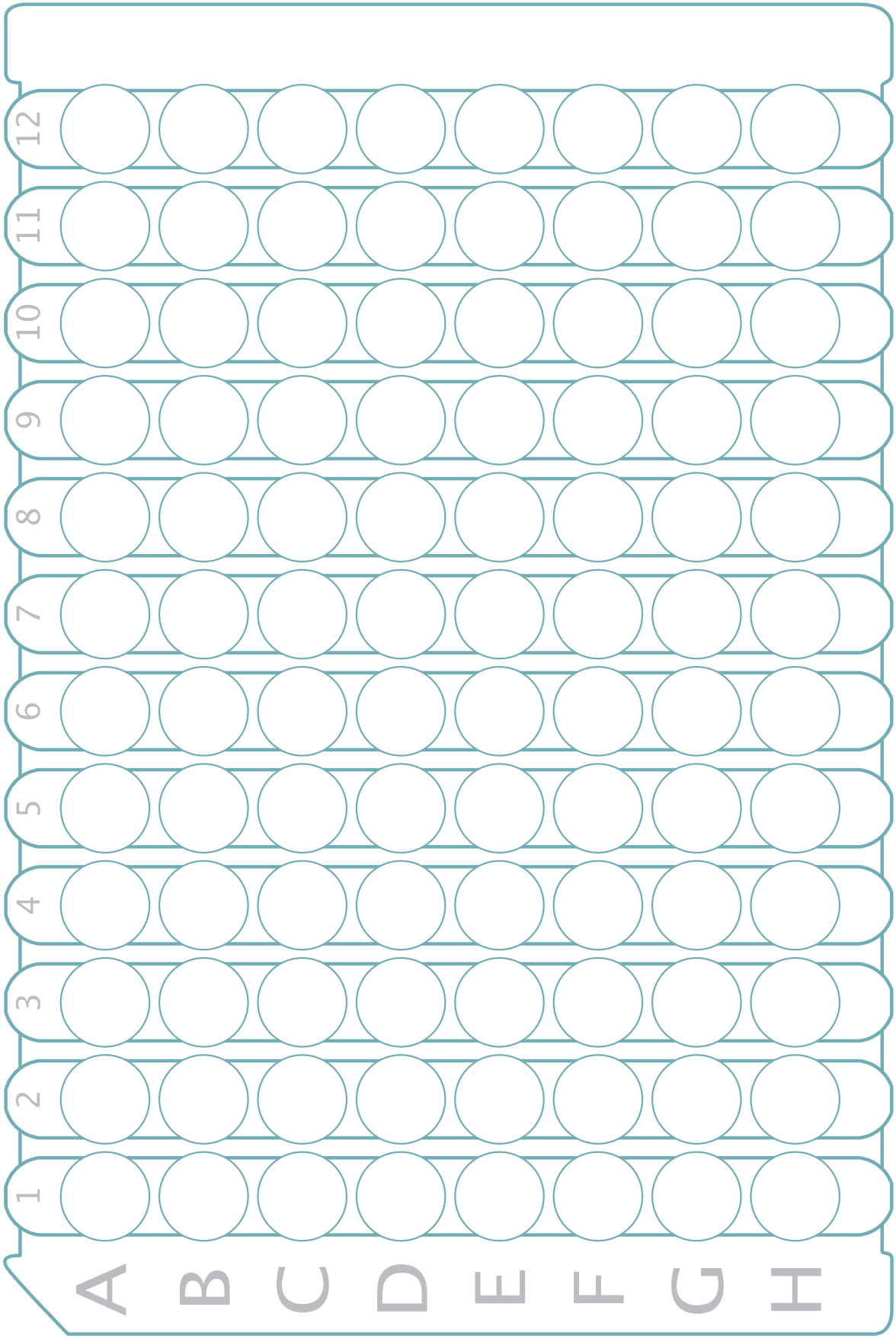
TREM-1

REFERENCES

1. Colonna, M. (2003) Nat. Rev. Immunol. **3**:1.
2. Nathan, C. and A. Ding (2001) Nat. Med. **7**:530.
3. Colonna, M. and F. Facchetti (2003) J. Inf. Dis. **187**(2):S397.
4. Chung, D-H. *et al.* (2002) Eur. J. Immunol. **32**:59.
5. Daws, M.R. *et al.* (2001) Eur. J. Immunol. **31**:783.
6. Allcock, R.J.N. *et al.* (2003) Eur. J. Immunol. **33**:567.
7. Bouchon, A. *et al.* (2000) J. Immunol. **164**:4991.
8. Lanier, L.L. *et al.* (1998) Nature **391**:703.
9. Kelker, M.S. *et al.* (2004) J. Mol. Biol. **344**:1175.
10. Gingras, M-C. *et al.* (2001) Mol. Immunol. **38**:817.
11. GenBank Accession #: XP_217336.
12. Gibot, S. *et al.* (2005) Crit. Care Med. **33**:1787.
13. Knapp, S. *et al.* (2004) J. Immunol. **173**:7131.
14. Bouchon, A. *et al.* (2001) Nature **410**:1103.
15. Gibot, S. *et al.* (2005) Crit. Care Med. **31**:594.
16. Bleharski, J.R. *et al.* (2003) J. Immunol. **170**:3812.
17. Gibot, S. *et al.* (2004) J. Exp. Med. **200**:1419.
18. Radsak, M.P. *et al.* (2004) J. Immunol. **172**:4956.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



For research use only. Not for use in diagnostic procedures.

NOTES

NOTES

All trademarks and registered trademarks are the property of their respective owners.

©2016 R&D Systems, Inc.