

# Quantikine<sup>®</sup>

## Human TREM-1 Immunoassay

Catalog Number DTRM10B

**For the quantitative determination of human Triggering Receptor Expressed on Myeloid Cells 1 (TREM-1) concentrations in cell culture supernates, serum, plasma, and saliva.**

*This package insert must be read in its entirety before using this product.*

 **フナコシ株式会社**

試薬に関して: Tel. 03-5684-1620 / Fax 03-5684-1775

e-mail: reagent@funakoshi.co.jp

2009/11/13

**FOR RESEARCH USE ONLY.  
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

# TABLE OF CONTENTS

Contents	Page
INTRODUCTION	2
PRINCIPLE OF THE ASSAY . . . . .	2
LIMITATIONS OF THE PROCEDURE	3
MATERIALS PROVIDED . . . . .	3
STORAGE	3
OTHER SUPPLIES REQUIRED . . . . .	4
PRECAUTIONS	4
SAMPLE COLLECTION AND STORAGE . . . . .	4
REAGENT PREPARATION	5
ASSAY PROCEDURE . . . . .	6
ASSAY PROCEDURE SUMMARY	7
CALCULATION OF RESULTS. . . . .	8
TYPICAL DATA	8
TECHNICAL HINTS . . . . .	9
PRECISION	9
RECOVERY . . . . .	10
LINEARITY	10
SENSITIVITY . . . . .	11
CALIBRATION	11
SAMPLE VALUES . . . . .	11
SPECIFICITY	13
REFERENCES . . . . .	14
PLATE LAYOUT	15

## MANUFACTURED AND DISTRIBUTED BY:

R&D Systems, Inc.	TELEPHONE:	(800) 343-7475
614 McKinley Place NE		(612) 379-2956
Minneapolis, MN 55413	FAX:	(612) 656-4400
United States of America	E-MAIL:	info@RnDSystems.com

## DISTRIBUTED BY:

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

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

## **INTRODUCTION**

The TREM (triggering receptor expressed on myeloid cells) gene cluster on 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 - 4). 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 (5, 6). In contrast, TLTs lack a transmembrane charged lysine residue but have a long cytoplasmic domain with one or more immunoreceptor tyrosine-based inhibitory motifs (ITIM) that transduce cellular inhibition signals (4).

Human TREM-1 is a 30 kDa monomeric transmembrane activating receptor that is synthesized as a 234 amino acid (aa) residue precursor with a 16 aa signal peptide, a 184 aa extracellular domain, a 29 aa transmembrane domain, and a short 5 aa cytoplasmic domain (5, 7). TREM-1 is expressed by neutrophils, monocytes, and various macrophage subsets (3, 5, 8, 19). During infection by Gram-positive and Gram-negative bacteria and fungi, the expression of membrane-bound TREM-1 is highly upregulated (9, 20). A soluble form of TREM-1 is also released from cells and can be measured in biological fluids (10, 11, 15 - 17, 19, 20).

TREM-1 is an important regulator in innate immunity and functions to amplify inflammation in response to infection (5, 9, 12, 13). The natural ligand for TREM-1 has not been identified. However, 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. Monocyte TREM-1 activation also induces the differentiation of primary monocytes into immature dendritic cells (12). On neutrophils, TREM-1 ligation induces degranulation, respiratory burst and phagocytosis synergistically with TLR ligands (13). Soluble TREM-1 is being investigated as a clinical marker to distinguish sepsis from non-infectious inflammatory conditions (14 - 17). Furthermore, in mouse models, blockade of TREM-1 was protective against septic shock, identifying TREM-1 as a potential target for therapeutic intervention in sepsis in humans (9).

The Quantikine Human TREM-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human TREM-1 in cell culture supernates, serum, plasma, and saliva. It contains NS0-expressed recombinant human TREM-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human TREM-1 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that the Quantikine Human TREM-1 kit can be used to determine relative mass values for naturally occurring TREM-1.

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TREM-1 has been pre-coated onto a microplate. Standards 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 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 and color develops in proportion to the amount of TREM-1 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 standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

**TREM-1 Microplate** (Part 893660) - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against TREM-1.

**TREM-1 Conjugate** (Part 893046) - 21 mL of polyclonal antibody against TREM-1 conjugated to horseradish peroxidase with preservatives.

**TREM-1 Standard** (Part 893047) - 40 ng of recombinant human TREM-1 in a buffered protein solution with preservatives; lyophilized.

**Assay Diluent RD1-90** (Part 895566) - 11 mL of a buffered protein solution with blue dye and preservatives.

**Calibrator Diluent RD5-18** (Part 895335) - 21 mL of a buffer with preservatives.

**Wash Buffer Concentrate** (Part 895003) - 21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives.

**Color Reagent A** (Part 895000) - 12.5 mL of stabilized hydrogen peroxide.

**Color Reagent B** (Part 895001) - 12.5 mL of stabilized chromogen (tetramethylbenzidine).

**Stop Solution** (Part 895032) - 6 mL of 2 N sulfuric acid.

**Plate Covers** - 4 adhesive strips.

## STORAGE

<b>Unopened Kit</b>	Store at 2 - 8° C. Do not use past kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1-90	
	Calibrator Diluent RD5-18	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	
Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*	

\*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.
- Human TREM-1 Controls (optional; available from R&D Systems).

## PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

TREM-1 is detectable in saliva. Precautionary measures should be taken to prevent the contamination of kit reagents while running this assay.

## SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

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

## REAGENT PREPARATION

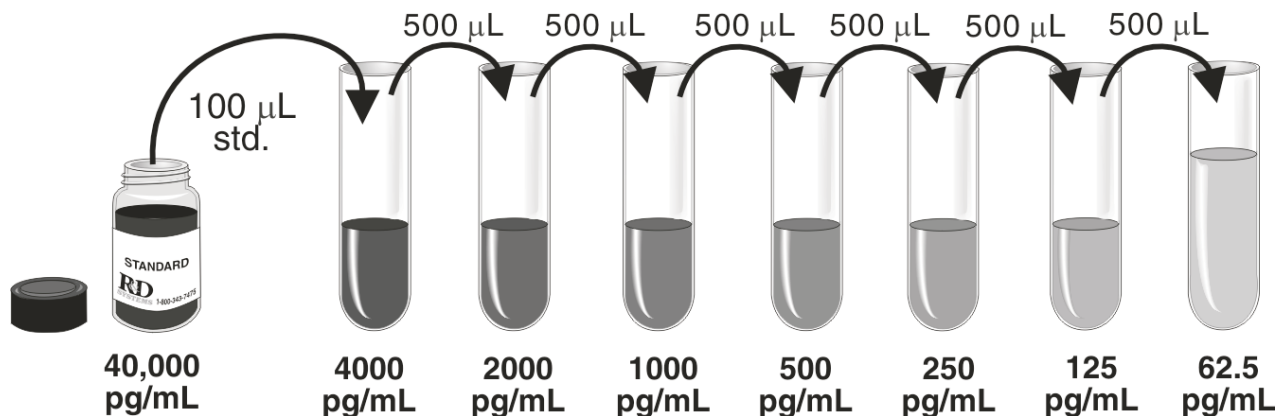
Bring all reagents to room temperature before use.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate into 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  $\mu\text{L}$  of the resultant mixture is required per well.

**TREM-1 Standard** - Reconstitute the TREM-1 Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900  $\mu\text{L}$  of Calibrator Diluent RD5-18 into the 4000 pg/mL tube. Pipette 500  $\mu\text{L}$  of Calibrator Diluent RD5-18 into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The Calibrator Diluent 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 samples, controls, and standards be assayed in duplicate.**

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 50  $\mu\text{L}$  of Assay Diluent RD1-90 to each well.
4. Add 50  $\mu\text{L}$  of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. 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  $\mu\text{L}$ ) 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  $\mu\text{L}$  of TREM-1 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50  $\mu\text{L}$  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.

## ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Add 50  $\mu\text{L}$  Assay Diluent RD1-90 to each well.



3. Add 50  $\mu\text{L}$  Standard, control, or sample to each well. Incubate 2 hrs. at RT.



4. Aspirate and wash 4 times.



5. Add 200  $\mu\text{L}$  Conjugate to each well. Incubate 2 hrs. at RT.



6. Aspirate and wash 4 times.



7. Add 200  $\mu\text{L}$  Substrate Solution to each well. Incubate 30 min. **Protect from light.**



8. Add 50  $\mu\text{L}$  Stop Solution to each well. Read at 450 nm within 30 min.  
 $\lambda$  correction 540 or 570 nm

## CALCULATION OF RESULTS

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

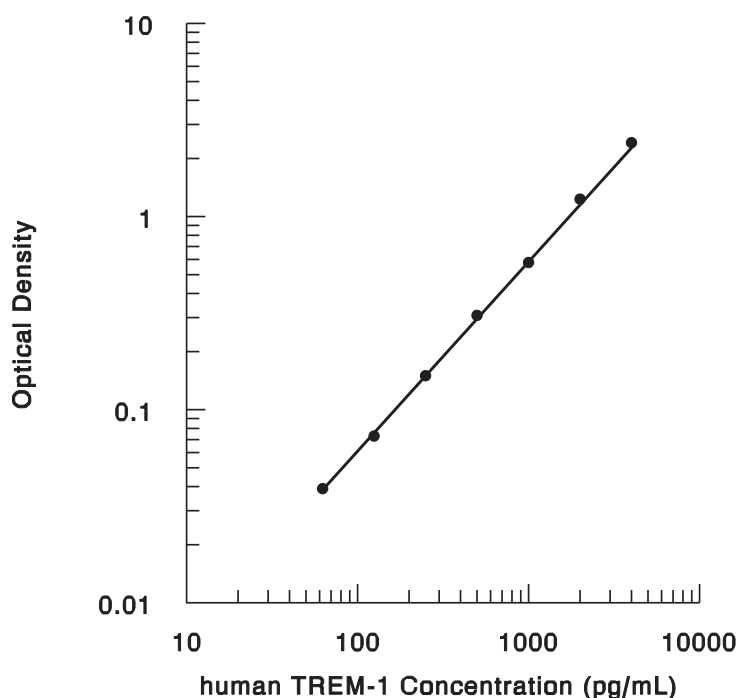
Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation.

To determine the TREM-1 concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding TREM-1 concentration.

If the 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.



<u>pg/mL</u>	<u>O.D.</u>	<u>Average</u>	<u>Corrected</u>
0	0.016 0.029 0.061	0.023	—
62.5	0.062 0.094	0.062	0.039
125	0.097 0.172	0.096	0.073
250	0.173 0.329	0.173	0.150
500	0.332 0.596	0.331	0.308
1000	0.608 1.246	0.602	0.579
2000	1.263 2.390	1.255	1.232
4000	2.486	2.438	2.415

## 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.
- 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.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## 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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	410	1212	2424	439	1215	2354
Standard deviation	21.3	50.7	87.9	32.7	70.4	166
CV (%)	5.2	4.2	3.6	7.4	5.8	7.1

## RECOVERY

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

Sample	Average % Recovery	Range
Cell culture media (n=4)	109	97 - 119%
Serum (n=4)	98	87 - 110%
Heparin plasma (n=4)	99	85 - 109%
EDTA plasma (n=4)	98	86 - 108%

## LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of TREM-1 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=5)	Heparin plasma (n=5)	EDTA plasma (n=5)
1:2	Average % of Expected	103	105	113	110
	Range (%)	95 - 111	93 - 113	103 - 120	105 - 115
1:4	Average % of Expected	106	109	115	113
	Range (%)	103 - 110	94 - 115	106 - 121	105 - 120
1:8	Average % of Expected	103	105	111	106
	Range (%)	101 - 105	89 - 119	98 - 120	100 - 110
1:16	Average % of Expected	99	93	98	99
	Range (%)	95 - 103	83 - 103	83 - 112	89 - 113

## SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of TREM-1 ranged from 3.88 - 30.6 pg/mL. The mean MDD was 13.8 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.

## CALIBRATION

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

## SAMPLE VALUES

**Serum/Plasma/Saliva** - Samples from apparently healthy volunteers were evaluated for the presence of TREM-1 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=35)	179	92.0 - 330	60.5
Heparin plasma (n=35)	134	63.4 - 134	40.6

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
EDTA plasma (n=35)	130	97	ND - 254
Saliva (n=10)	271	89	ND - 702

ND = Non-detectable

### Cell Culture Supernates -

Human peripheral blood leukocytes (PBLs;  $1 \times 10^6$  cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 5  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of natural TREM-1.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	77.2	103
Stimulated	153	426

Human monocytes ( $2.5 \times 10^6$  cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. Cells were unstimulated or stimulated with 25 ng/mL rhGM-CSF, incubated for 6 days, and treated with 50 ng/mL lipopolysaccharide (LPS) on the final day. Aliquots of the cell culture supernate were removed and assayed for levels of natural TREM-1.

Condition	Observed Levels (pg/mL)
Unstimulated	87.7
Stimulated	1145

THP-1 cells were grown to 60% confluency in RPMI supplemented with 10% fetal calf serum and 50  $\mu$ M  $\beta$ -mercaptoethanol. Cells were treated with 1  $\mu$ g/mL rhIFN- $\gamma$  for 8 hours followed by an overnight stimulation with 1  $\mu$ g/mL LPS. Cells stimulated with only LPS were treated for 24 hours.

Condition	Observed Levels (pg/mL)
Unstimulated	ND
Stimulated + LPS	117
Stimulated + LPS + IFN- $\gamma$	ND

ND = Non-detectable

U937 cells were cultured in RPMI supplemented with 10% fetal calf serum and 2 mM L-glutamine. Cells were differentiated with the addition of 250 ng/mL  $1\alpha,25$ -dihydroxy vitamin D3 and 1 ng/mL rhTGF- $\beta$ 1 for 4 days. Treated cells were stimulated with 1  $\mu$ g/mL LPS for 4 days. Aliquots of the cell culture supernate were removed and assayed for levels of natural TREM-1.

Condition	Observed Levels (pg/mL)
Differentiated	1236
Differentiated + LPS	1674

## SPECIFICITY

This assay recognizes recombinant and natural human 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 recombinant human TREM-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant human:

Amphiregulin	IL-15
Angiogenin	IL-16
Angiopoietin-2	IL-17
BDNF	IL-19
Cardiotropin-1	IL-20
CD4	IL-22
CD40	IL-24
CD40 Ligand	IL-26 monomer
CNTF	IL-26 dimer
CTLA-4	IL-28A
Epo	IL-29
Fas	Leptin
GDNF	LIF
GITR	MIF
GITR Ligand	MK
IFN- $\gamma$	NK p44
IL-1 $\alpha$	NT-3
IL-1 $\beta$	NT-4
IL-1ra	OPG
IL-2	OSM
IL-3	PTN
IL-4	SCF
IL-5	SLPI
IL-6	SMDF
IL-7	TREML1/TLT-1
IL-8	TNF- $\alpha$
IL-9	TNF- $\beta$
IL-10	Tpo
IL-11	TRAIL
IL-12	TRANCE
IL-12 p40	TREM-2
IL-13	

### Recombinant mouse:

Cardiotropin-1
CTLA-4
Fas
Fas Ligand
IFN- $\gamma$
IL-1 $\alpha$
IL-1 $\beta$
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-11
IL-12
IL-12 p40
IL-13
IL-17
Leptin
LIF
OPG
OPN
OSM
SCF
TNF- $\alpha$
Tpo
TRANCE
TREM-1
TREM-2b
TREM-3

### Recombinant rat:

CNTF
GDNF
IFN- $\gamma$
IL-1 $\alpha$
IL-1 $\beta$
IL-2
IL-4
IL-6
IL-10
Leptin
TNF- $\alpha$

### Recombinant porcine:

IL-1 $\alpha$
IL-1 $\beta$
IL-2
IL-4
IL-6
IL-8
IL-10
TNF- $\alpha$

## 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**:S397.
4. Allcock, R.J.N. *et al.* (2003) *Eur. J. Immunol.* **33**:567.
5. Bouchon, A. *et al.* (2000) *J. Immunol.* **164**:4991.
6. Lanier, L.L. *et al.* (1998) *Nature* **391**:703.
7. Kelker, M.S. *et al.* (2004) *J. Mol. Biol.* **344**:1175.
8. Knapp, S. *et al.* (2004) *J. Immunol.* **173**:7131.
9. Bouchon, A. *et al.* (2001) *Nature* **410**:1103.
10. Gibot, S. *et al.* (2004) *J. Exp. Med.* **200**:1419.
11. Gibot, S. *et al.* (2005) *Int. Care Med.* **31**:594.
12. Bleharski, J.R. *et al.* (2003) *J. Immunol.* **170**:3812.
13. Radsak, M.P. *et al.* (2004) *J. Immunol.* **172**:4956.
14. Gibot, S. and A. Cravoisy (2004) *Clin. Med. Res.* **2**:181.
15. Gibot, S. *et al.* (2004) *Annals of Internal Medicine* **141**:9.
16. Gibot, S. *et al.* (2004) *New England J. Med.* **350**:451.
17. Determann, R.M. *et al.* (2005) *Intensive Care Medicine* **31**:1495.
18. Koussoulas, V. *et al.* (2006) *Eur. J. Gastroenterol. Hepatol.* **18**:375.
19. Gringas, M. *et al.* (2001) *Mol. Immunol.* **38**:817.
20. Begum, N.A. *et al.* (2004) *Infect. Immunol.* **72**:937.

# PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

12									
11									
10									
9									
8									
7									
6									
5									
4									
3									
2									
1									
	A	B	C	D	E	F	G	H	