bio-techne®

R&D SYSTEMS

Quantikine™ ELISA

Mouse TNF-α Immunoassay

Catalog Number MTA00B-1
SMTA00B
PMTA00B

For the quantitative determination of mouse Tumor Necrosis Factor alpha (TNF-α) 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

Tumor necrosis factor alpha (TNF-α), also known as cachectin and TNFSF1A, is the prototypic ligand of the TNF superfamily (1). It is a pleiotropic molecule that plays a central role in inflammation, immune system development, apoptosis, and lipid metabolism (2-5). TNF-α is also involved in a number of pathological conditions including asthma, Crohn's disease, rheumatoid arthritis, neuropathic pain, obesity, type 2 diabetes, septic shock, autoimmunity, and cancer (5-11).

Mouse TNF-α is synthesized as a 26 kDa type II transmembrane protein that consists of a 35 amino acid (aa) cytoplasmic domain, a 21 aa transmembrane segment, and a 179 aa extracellular domain (ECD) (12). Within the ECD, mouse TNF-α shares 95% aa identity with rat, and 80% aa identity with canine, equine, feline, human, rabbit, and porcine TNF-α. It is produced by a wide variety of immune, epithelial, endothelial, and tumor cells. TNF-α is assembled intracellularly to form a noncovalently linked homotrimer which is expressed on the cell surface (13). Cell surface TNF-α can both induce the lysis of tumor cells and virus infected cells, and generate its own downstream cell signaling following ligation by soluble TNF RI (14, 15). Shedding of membrane bound TNF-α by TACE/ADAM17 releases the bioactive cytokine, a 55 kDa soluble trimer containing the TNF-α extracellular domain (16-18).

TNF-α binds the ubiquitous 55-60 kDa TNF RI (19, 20) and the hematopoietic cell-restricted 78-80 kDa TNF RII (21, 22), both of which are also expressed as homotrimers (1, 23). Both type I and type II receptors bind TNF-α with comparable affinity and can promote NFkB activation (24-27). Only TNF RI, however, contains a cytoplasmic death domain which triggers the activation of apoptosis (3, 28). Soluble forms of both types of receptors are released into human serum and urine, and can neutralize the biological activity of TNF (29-31).

The Quantikine™ Mouse TNF-α Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse TNF-α levels in cell culture supernates, serum, and plasma. It contains recombinant mouse TNF-α and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse TNF-α accurately. Results obtained using natural mouse TNF-α 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 natural mouse TNF-α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse TNF-α has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any TNF-α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse TNF-α 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 TNF-α 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 the appropriate 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 #	CATALOG # MTA00B-1	CATALOG # SMTA00B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse TNF-α Microplate	893961	1 plate	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse TNF-α.	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 TNF-α Standard	893963	2 vials	6 vials	Recombinant mouse TNF-α in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse TNF-α Control	893964	2 vials	6 vials	Recombinant mouse TNF-α in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse TNF-α Conjugate	899286	1 vial	6 vials	12.5 mL/vial of a polyclonal antibody specific for mouse TNF-α conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-63	895352	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	1 vial	3 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-12	895214	1 vial	3 vials	21 mL/vial of a buffered protein base with preservatives. *For serum/plasma samples.*	
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	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

MTA00B-1 contains sufficient materials to run ELISAs on one 96 well plate.
SMTA00B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PMTA00B). 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®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Mouse TNF-α Microplate	893961	50 plates
Mouse TNF-α Conjugate	899286	50 vials
Mouse TNF-α Standard*	893963	25 vials
Mouse TNF-α Control	893964	25 vials
Calibrator Diluent RD5K	895119	25 vials
or		
Calibrator Diluent RD6-12	895214	25 vials
Assay Diluent RD1-63	895352	25 vials
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Wash Buffer Concentrate	895126	9 bottles
Stop Solution	895174	25 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
- 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. 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 - 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: *Grossly hemolyzed samples are not suitable for use in this assay.*
Citrate plasma has not been validated for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

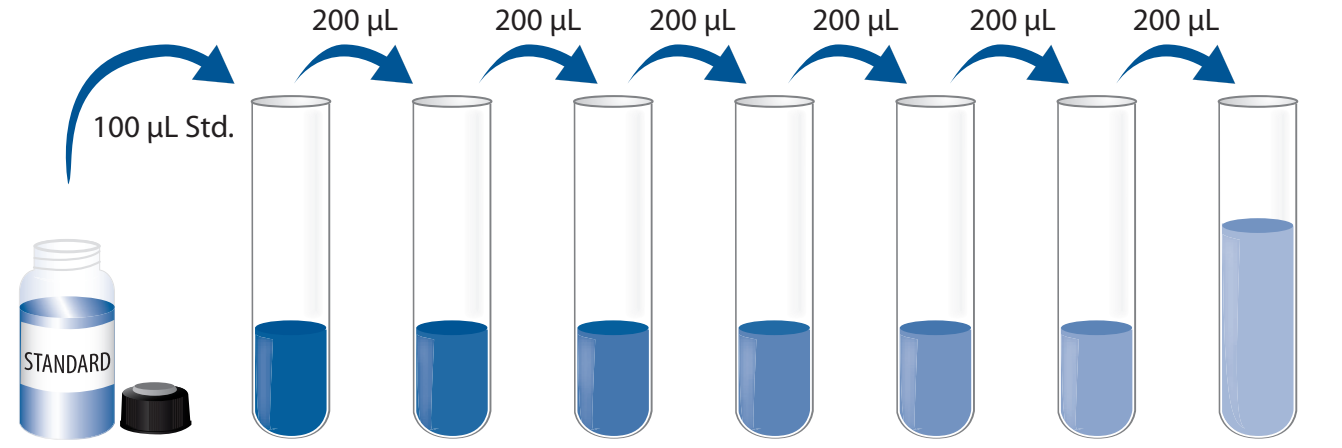
Mouse TNF-α Control - Reconstitute the control with 1 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 into 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.

Mouse TNF-α Standard -**Refer to the vial label for standard reconstitution volume.** Reconstitute the Mouse TNF-α Standard with deionized or distilled water. This reconstitution produces a stock solution of 7000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5K *(for cell culture supernate samples)* or Calibrator Diluent RD6-12 *(for serum/plasma samples*) into the 700 pg/mL tube. Pipette 200 µL of the appropriate calibrator diluent in the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 700 pg/mL standard serves as the high standard. The appropriate 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 standards, control, and samples be assayed in duplicate.

1. Prepare reagents, samples, and standard dilutions 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 RD1-63 to each well.
4. Add 50 µL of standard, control, or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided as a record of samples and standards 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 TNF-α 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **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.

CALCULATION OF RESULTS

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

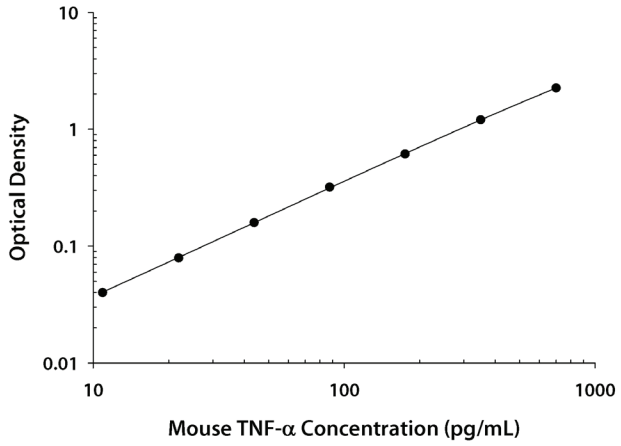
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

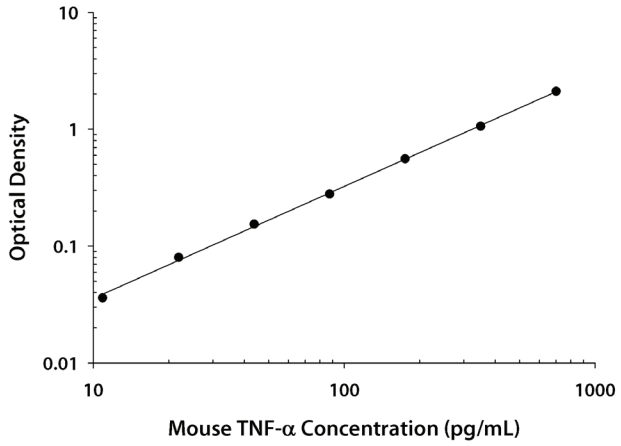
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.020 0.021	0.021	—
10.9	0.060 0.061	0.061	0.040
21.9	0.099 0.101	0.100	0.079
43.8	0.179 0.181	0.180	0.159
87.5	0.336 0.344	0.340	0.319
175	0.632 0.637	0.635	0.614
350	1.203 1.242	1.223	1.202
700	2.265 2.281	2.273	2.252

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.013 0.016	0.015	—
10.9	0.051 0.051	0.051	0.036
21.9	0.091 0.099	0.095	0.080
43.8	0.167 0.171	0.169	0.154
87.5	0.285 0.302	0.294	0.279
175	0.571 0.574	0.573	0.558
350	1.057 1.090	1.074	1.059
700	2.111 2.139	2.125	2.110

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.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	54.6	116	405	52.6	138	373
Standard deviation	1.50	4.51	12.5	4.61	8.49	29.7
CV (%)	2.7	3.9	3.1	8.8	6.2	8.0

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	50.5	134	375	53.0	146	388
Standard deviation	1.91	4.95	11.5	3.69	8.42	29.7
CV (%)	3.8	3.7	3.1	7.0	5.8	7.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	104	94-111%
Mouse serum (n=4)	94	88-106%
EDTA plasma (n=4)	95	85-103%
Heparin plasma (n=4)	92	86-98%

LINEARITY

To assess the linearity of the assay, samples were spiked with various concentrations of mouse TNF-α in each matrix, diluted with the appropriate calibrator diluent and then assayed.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	106	103	104	105
	Range (%)	102-110	90-111	102-105	98-108
1:4	Average % of Expected	105	105	105	110
	Range (%)	98-112	92-110	101-109	105-115
1:8	Average % of Expected	100	107	107	112
	Range (%)	89-113	99-111	101-113	110-115
1:16	Average % of Expected	105	107	109	109
	Range (%)	99-110	97-112	101-115	101-117

SENSITIVITY

One hundred seventeen assays were evaluated and the minimum detectable dose (MDD) of mouse TNF-α ranged from 0.36-7.21 pg/mL. The mean MDD was 1.88 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 recombinant mouse TNF-α produced at R&D Systems®.

The Non WHO Reference Material 88/532 was evaluated in this kit. Each ampule contains a nominal 1 µg of recombinant mouse TNF-α, and was assigned an arbitrary unitage of 200,000 U/ampule. To convert sample values obtained with the Quantikine™ Mouse TNF-α kit to approximate NIBSC units, use the equation below:

NIBSC/WHO (88/532) approximate value (U/mL) = 1.8553 x Quantikine Mouse TNF-α (pg/mL)

Note: *Based on data generated in March 2017.*

SAMPLE VALUES

Serum/Plasma - Twenty individual mouse serum and plasma samples were evaluated for levels of mouse TNF-α in this assay. All samples measured less than the lowest Mouse TNF-α Standard, 10.9 pg/mL.

Cell Culture Supernates:
EL-4 mouse lymphoblast cells (2 x 10^5 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL lipopolysaccharide (LPS) and 100 ng/mL recombinant mouse (rm) IL-10 for 4 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse TNF-α.

Condition	(pg/mL)
Unstimulated	ND
Stimulated	1220

ND=Non-detectable

RAW 264.7 mouse monocyte/macrophage cells (3.5 x 10^6 cells/mL) were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 5.0 µg/mL LPS and 100 ng/mL rmIL-10 for 7 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse TNF-α.

Condition	(pg/mL)
Unstimulated	151
Stimulated	9605

Organs from individual mice were removed, rinsed in 1X PBS and kept on ice in tubes containing 1X PBS. Organs were cut into 1-2 mm pieces and homogenized using a tissue homogenizer. Cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 1.0 µg/mL LPS for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of mouse TNF-α.

Tissue Type	Unstimulated (pg/mL)	Stimulated (pg/mL)
Mouse Liver	ND	25.7
Mouse Lung	55.9	97.3
Mouse spleen	ND	34.6

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant mouse TNF-α.

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

Recombinant mouse:

CD40
CD40 Ligand
Fas
Fas Ligand
LIF
OPG
RANK
RANK-L
TNF-β
TRAIL
TROY

Recombinant human:

TNF-α

Recombinant canine:

TNF-α

Recombinant porcine:

TNF-α

Recombinant rat TNF-α cross-reacts approximately 47% in this assay.

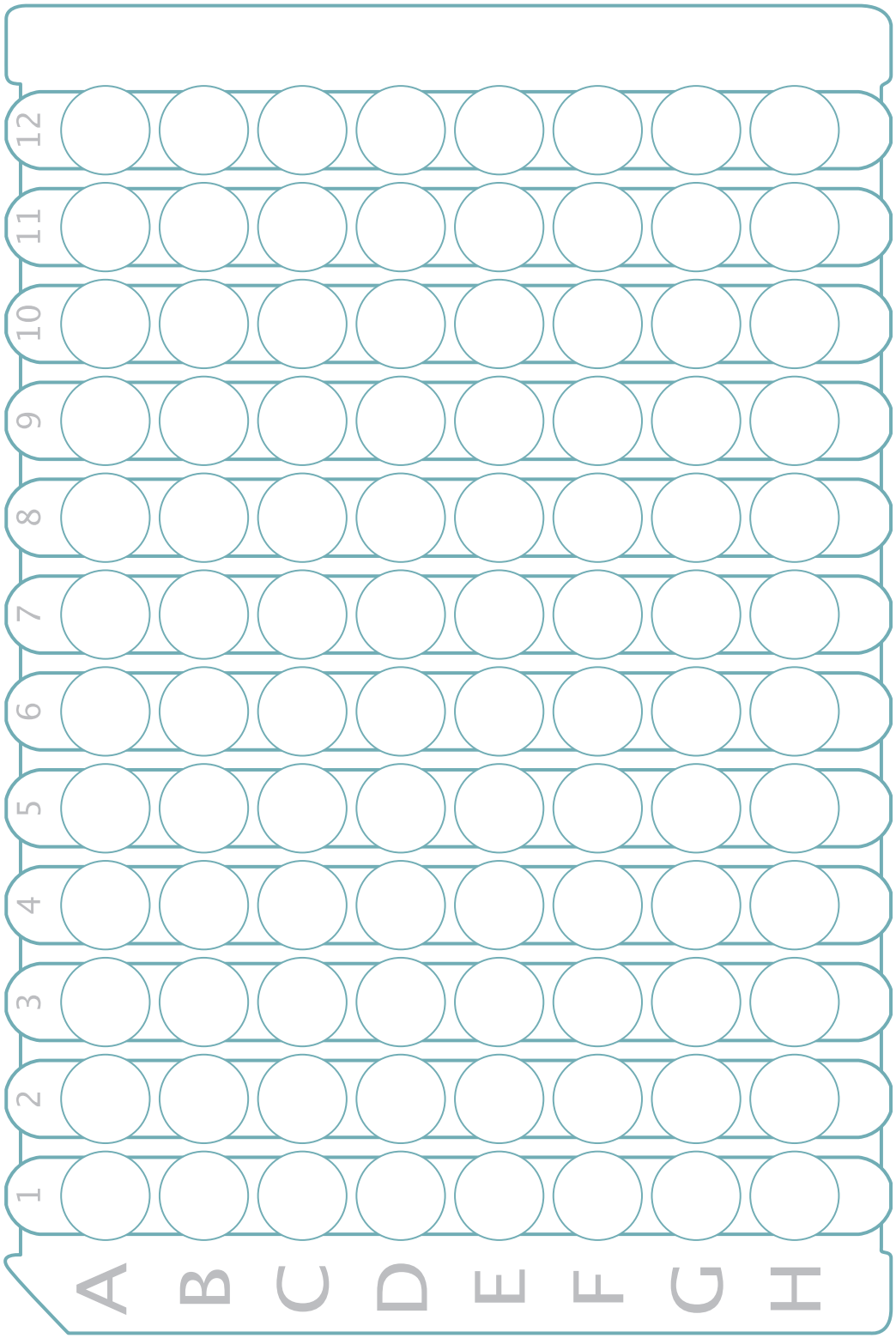
Recombinant mouse TNF RI and TNF RII were found to interfere with the measurement of TNF-α above concentrations of 1.25 ng/mL and 12.5 ng/mL respectively.

REFERENCES

1. Idriss, H.T. and J.H. Naismith (2000) Microsc. Res. Tech. **50**:184.
2. Salek-Ardakani, S. and M. Croft (2010) J. Interferon Cytokine Res. **30**:205.
3. Van Herreweghe, F. *et al.* (2010) Cell. Mol. Life Sci. **67**:1567.
4. Chen, X. *et al.* (2009) Cell Biochem. Funct. **27**:407.
5. Hehlgans, T. and K. Pfeffer (2005) Immunology **115**:1.
6. Berry, M. *et al.* (2007) Curr. Opin. Pharmacol. **7**:279.
7. D'Haens, G. (2003) Curr. Pharm. Des. **9**:289.
8. Feldmann, M. and R.N. Maini (2001) Annu. Rev. Immunol. **19**:163.
9. Leung, L. and C.M. Cahill (2010) J. Neuroinflammation **7**:27.
10. Tzanavari, T. *et al.* (2010) Curr. Dir. Autoimmun. **11**:145.
11. Wu, Y. and B.P. Zhou (2010) Br. J. Cancer **102**:639.
12. Pennica, D. *et al.* (1984) Nature **312**:724.
13. Tang, P. *et al.* (1996) Biochemistry **35**:8216.
14. Perez, C. *et al.* (1990) Cell **63**:251.
15. Watts, A.D. *et al.* (1999) EMBO J. **18**:2119.
16. Black, R.A. *et al.* (1997) Nature **385**:729.
17. Moss, M.L. *et al.* (1997) Nature **385**:733.
18. Gearing, A.J.H. *et al.* (1994) Nature **370**:555.
19. Schall, T.J. *et al.* (1990) Cell **61**:361.
20. Loetscher, H. *et al.* (1990) Cell **61**:351.
21. Dembic, Z. *et al.* (1990) Cytokine **2**:231.
22. Smith, C.A. *et al.* (1990) Science **248**:1019.
23. Loetscher, H. *et al.* (1991) J. Biol. Chem. **266**:18324.
24. Rothe, M. *et al.* (1995) Science **269**:1424.
25. Ruby, J. *et al.* (1997) J. Exp. Med. **186**:1591.
26. Pinckard, J.K. *et al.* (1997) J. Biol. Chem. **272**:10784.
27. Mukhopadhyay, A. *et al.* (2001) J. Biol. Chem. **276**:31906.
28. Hsu, H. *et al.* (1995) Cell **81**:495.
29. Seckinger, P. *et al.* (1989) J. Biol. Chem. **264**:11966.
30. Olsson, I. *et al.* (1989) Eur. J. Haematol. **42**:270.
31. Engelmann, H. *et al.* (1990) J. Biol. Chem. **265**:1531.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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