Quantikine® ELISA

Human TNF RII/TNFRSF1B Immunoassay

Catalog Number DRT200
SRT200
PDRT200

For the quantitative determination of human Tumor Necrosis Factor Receptor II (TNF RII) concentrations in cell culture supernates, serum, plasma, and urine.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
LIMITATIONS OF THE PROCEDURE	3
TECHNICAL HINTS	3
PRECAUTIONS	3
MATERIALS PROVIDED & STORAGE CONDITIONS	4
OTHER SUPPLIES REQUIRED	5
SAMPLE COLLECTION & STORAGE	5
SAMPLE PREPARATION	5
REAGENT PREPARATION	6
ASSAY PROCEDURE	7
CALCULATION OF RESULTS	8
TYPICAL DATA	8
PRECISION	9
RECOVERY	9
SENSITIVITY	9
LINEARITY	10
CALIBRATION	10
SAMPLE VALUES	10
SPECIFICITY	11
REFERENCES	12
PLATE LAYOUT	13

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

Tumor necrosis factors (TNFs) are pleiotropic cytokines that are considered primary modifiers of the inflammatory and immune reactions of animals produced in response to injury or infection. Two forms of TNF, designated TNF-α (or cachectin) and TNF-β (or lymphotoxin), have been described that share 30% sequence similarity and compete for binding to the same receptors. TNFs play a necessary and beneficial role as mediators of host resistance to infections and tumor formation, however, over-production or inappropriate expression of these factors can lead to a variety of pathological conditions, including wasting, systemic toxicity, and septic shock. For reviews of the literature relating to these factors, see references 1 and 2.

The actions of TNFs are produced subsequent to binding to cell surface receptors. Two distinct TNF receptors have been identified and cloned. Virtually all cell types studied show the presence of one or both of these receptor types. One receptor type, termed TNF RII (or Type A, Type α, 75 kDa, or utr antigen), shows an apparent molecular weight of 75 kDa. The gene for this receptor encodes a presumptive transmembrane protein of 439 amino acid (aa) residues (3,19). The other receptor type, termed TNF RI (or Type B, Type β, 55 kDa or htr antigen), shows an apparent molecular weight of 55 kDa. The gene for this protein encodes a transmembrane protein of 426 aa residues (4, 5, 19). Both receptor types show high affinity binding of either TNF-α or TNF-β. The two receptor types are immunologically distinct but their extracellular domains show similarities in the pattern of cysteine residue locations in four domains (3). The intracellular domains of the two receptor types are apparently unrelated, suggesting the possibility that the two receptor types employ different signal transduction pathways. Alternatively, an additional, as yet unidentified, intracellular factor could be involved in mediating the signal transduction pathways.

Several groups have identified soluble TNF binding proteins in human serum and urine (6-8) that can neutralize the biological activities of TNF-α and TNF-β. Two types have been identified and designated sTNF RI (or TNF BPI) and sTNF RII (or TNF BPII). These soluble forms have now been shown to represent truncated forms of the two types of TNF receptors discussed above. The soluble receptor forms apparently arise as a result of shedding of the extracellular domains of the receptors, and concentrations of about 1-2 ng/mL are found in the serum and urine of healthy subjects (9, 10). The levels of the soluble receptors vary from individual to individual but are stable over time for given individuals (9).

Elevated levels of TNF Rs have been found in the amniotic fluid and urine of pregnant women (11), in serum or plasma in association with pathological conditions such as endotoxinemia, meningiococcemia, and HIV infection (12-15) and in plasma and ascites of patients in association with infections and malignancies (16). The mechanisms involved in the induction of shedding of the TNF receptors are not well understood. There are reports of correlations between increased TNF levels and soluble receptor levels, suggesting generally that stimuli that cause TNF levels to rise also induce shedding of TNF receptors (12-14, 17). There is also evidence, however, that suggests that the shedding of the two types of soluble receptors are independently regulated (13).

The physiological role of the soluble TNF receptors is not known. It is known that both types of soluble receptors can bind to TNF *in vitro* and inhibit its biological activity by competing with cell surface receptors for TNF binding. Consequently, it has been suggested that shedding of soluble receptors in response to TNF release could serve as a mechanism for binding and inhibiting the TNF not immediately bound to surface receptors, thus protecting other cells from the effects of TNF and localizing the inflammatory response (12, 17). It is also possible that shedding of receptors represents a mechanism for desensitizing the cells that shed the receptors from the effects of TNF (17). On the other hand, it has been reported that at low concentrations of TNF, binding to soluble receptors can stabilize TNF and augment some of its activities (18). Thus it is possible that under some conditions the pool of TNF bound to soluble receptors could represent a reservoir for the stabilization and controlled release of TNF.

The Quantikine® Human TNF RII Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure human TNF RII in cell culture supernates, serum, plasma, or urine. It contains *E. coli*-expressed recombinant human TNF RII as well as antibodies raised against this polypeptide. The recombinant protein represents the non-glycosylated, N-terminal methionyl form of the natural human soluble Type II receptor for TNF, minus 53 amino acids from the proline-rich region of the protein just exterior to the transmembrane domain, with an apparent molecular weight of approximately 19.8 kDa. This immunoassay has been shown to accurately quantitate the recombinant TNF RII. Results obtained using natural TNF RII 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 human TNF RII. Since the measurement of human TNF RII by this immunoassay is insensitive to added TNF-α or TNF-β, it is probable that this measurement corresponds to the total amount of the soluble receptor present in samples, *i.e.*, the total amount of free receptor plus the total amount of receptor bound to TNF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TNF RII has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF RII present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human TNF RII 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 TNF RII 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, further 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

PRECAUTIONS

Some components of this kit contain sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 MSDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DRT200	CATALOG # SRT200	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human TNF RII Microplate	890120	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human TNF RII.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human TNF RII Conjugate	890121	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human TNF RII conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human TNF RII Standard	890122	1 vial	6 vials	Recombinant human TNF RII in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-6	895158	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-5	895485	2 vials	12 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate/urine samples.*	
Calibrator Diluent RD6O	895120	2 vials	12 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples. May contain a precipitate. Mix well before and during use.*	
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	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DRT200 contains sufficient materials to run an ELISA on one 96 well plate.
SRT200 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDRT200). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- **Polypropylene** test tubes for dilution of standards and samples.
- Human TNF RII Controls (R&D Systems®, Catalog # QC05).

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Note: *Slight variations in measured values may occur between samples prepared by different procedures. It is recommended that comparisons be made within a single sample type.*

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

SAMPLE PREPARATION

Warning: Polypropylene tubes must be used. Do not use glass.

Serum and plasma samples require at least a 10-fold dilution prior to assay.
A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD6O.

Urine samples require at least a 10-fold dilution prior to assay. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5-5.

REAGENT PREPARATION

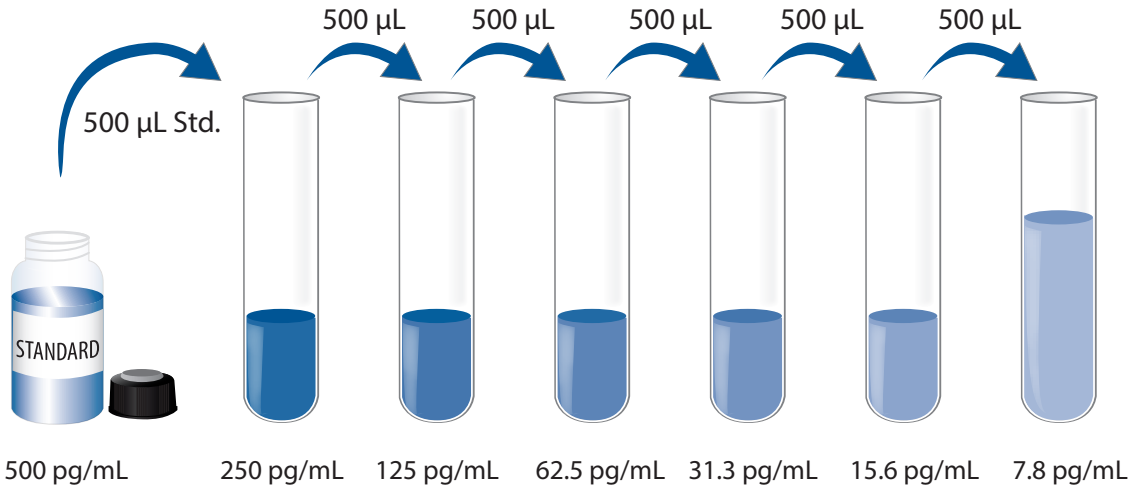
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. 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. 200 µL of the resultant mixture is required per well.

Human TNF RII Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human TNF RII Standard with Calibrator Diluent RD5-5 (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6O (*for serum/plasma samples*). This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 500 µL of Calibrator Diluent RD5-5 (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6O (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human TNF RII Standard (500 pg/mL) 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, samples, and controls be assayed in duplicate.

1. Prepare all reagents and working standards 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 RD1-6 to each well. *Assay Diluent RD1-6 may contain a precipitate. Mix well before and during its use.*
4. Add 200 µL 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 twice for a total of three 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 toweling.
6. Add 200 µL of Human TNF RII Conjugate to each well. Cover with a new adhesive strip. **For Cell Culture Supernates/Urine Samples:** Incubate for 1 hour at room temperature. **For Serum/Plasma Samples:** Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
9. Add 50 µL 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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

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

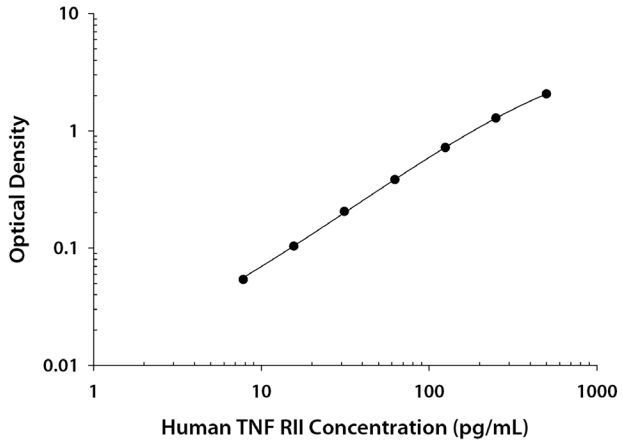
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

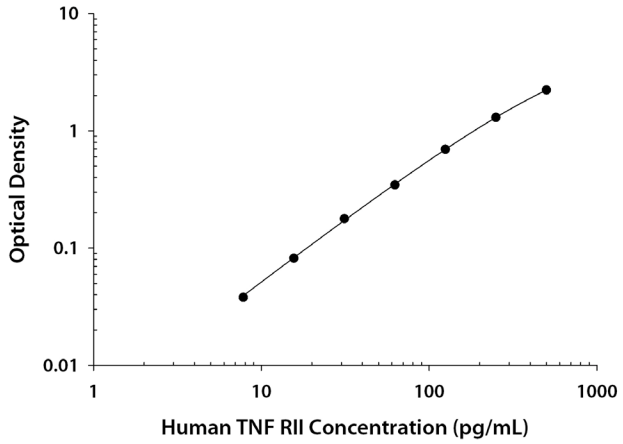
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.031 0.032	0.032	—
7.8	0.082 0.089	0.086	0.054
15.6	0.131 0.140	0.136	0.104
31.3	0.234 0.240	0.237	0.205
62.5	0.410 0.421	0.416	0.384
125	0.733 0.771	0.752	0.720
250	1.300 1.330	1.315	1.283
500	2.077 2.110	2.094	2.062

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.037 0.039	0.038	—
7.8	0.075 0.078	0.076	0.038
15.6	0.119 0.120	0.120	0.082
31.3	0.215 0.216	0.216	0.178
62.5	0.370 0.398	0.384	0.346
125	0.721 0.748	0.734	0.966
250	1.332 1.345	1.338	1.300
500	2.233 2.297	2.265	2.227

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/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	45.3	126	254	97.1	199	430
Standard deviation	2.0	3.7	10.4	3.1	6.9	17.8
CV (%)	4.4	2.9	4.1	3.2	3.5	4.1

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)	68.7	179	349	89.8	197	444
Standard deviation	2.2	4.6	16.8	4.6	6.9	16.2
CV (%)	3.2	2.6	4.8	5.1	3.5	3.6

RECOVERY

The recovery of human TNF RII spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	96	88-103%
Serum (n=4)	97	86-107%
EDTA plasma (n=4)	95	85-100%
Heparin plasma (n=4)	89	74-108%
Citrate plasma (n=4)	100	91-116%
Urine (n=4)	97	83-111%

SENSITIVITY

Ninety assays were evaluated and the minimum detectable dose (MDD) of human TNF RII ranged from 0.2-2.3 pg/mL. The mean MDD was 0.6 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.

LINEARITY

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

		Cell culture samples (n=4)	Serum* (n=8)	EDTA plasma* (n=8)	Heparin plasma* (n=8)	Citrate plasma* (n=8)	Urine* (n=8)
1:2	Average % of Expected	99	102	100	100	98	98
	Range (%)	97-104	94-109	89-113	96-110	93-103	91-104
1:4	Average % of Expected	100	100	97	98	95	97
	Range (%)	99-102	90-116	89-104	87-107	90-100	92-104
1:8	Average % of Expected	102	96	96	98	94	96
	Range (%)	100-107	88-103	92-101	91-107	87-101	92-102
1:16	Average % of Expected	106	95	93	93	93	94
	Range (%)	101-109	82-120	88-103	80-101	85-101	81-102

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

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human TNF RII produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human TNF RII 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=40)	1726	1003-3170	387
EDTA plasma (n=40)	1500	829-2262	320
Heparin plasma (n=40)	1678	951-2503	349
Citrate plasma (n=40)	1405	787-2145	286
Urine* (n=27)	1724	275-6982	1274

*The urine values are actual and are not normalized for creatinine content.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated with 10 µg/mL of PHA. Aliquots were removed on days 1, 3 and 5 and assayed.

Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
1841	2861	3124

SPECIFICITY

This assay recognizes natural and recombinant human TNF RII.

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

Recombinant human:

ANG
β-ECGF
FGF acidic
FGF basic
FGF-4
G-CSF
GM-CSF
GROα
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-6 R
IL-7
IL-8
IL-9
IL-10
IL-11
LIF
M-CSF
MCP-1
MIP-1α
MIP-1β
OSM
PDGF-AA
PDGF-AB
PDGF-BB
RANTES
SLPI
TGF-β1
TGF-β3
TGF-α
TNF-α
TNF-β
TNF RI

Recombinant mouse:

EGF
GM-CSF
IL-1β
IL-3
IL-4
IL-5
IL-7
IL-9
MIP-1α
MIP-1β
TNF-α
TNF RI
TNF RII

Other recombinants:

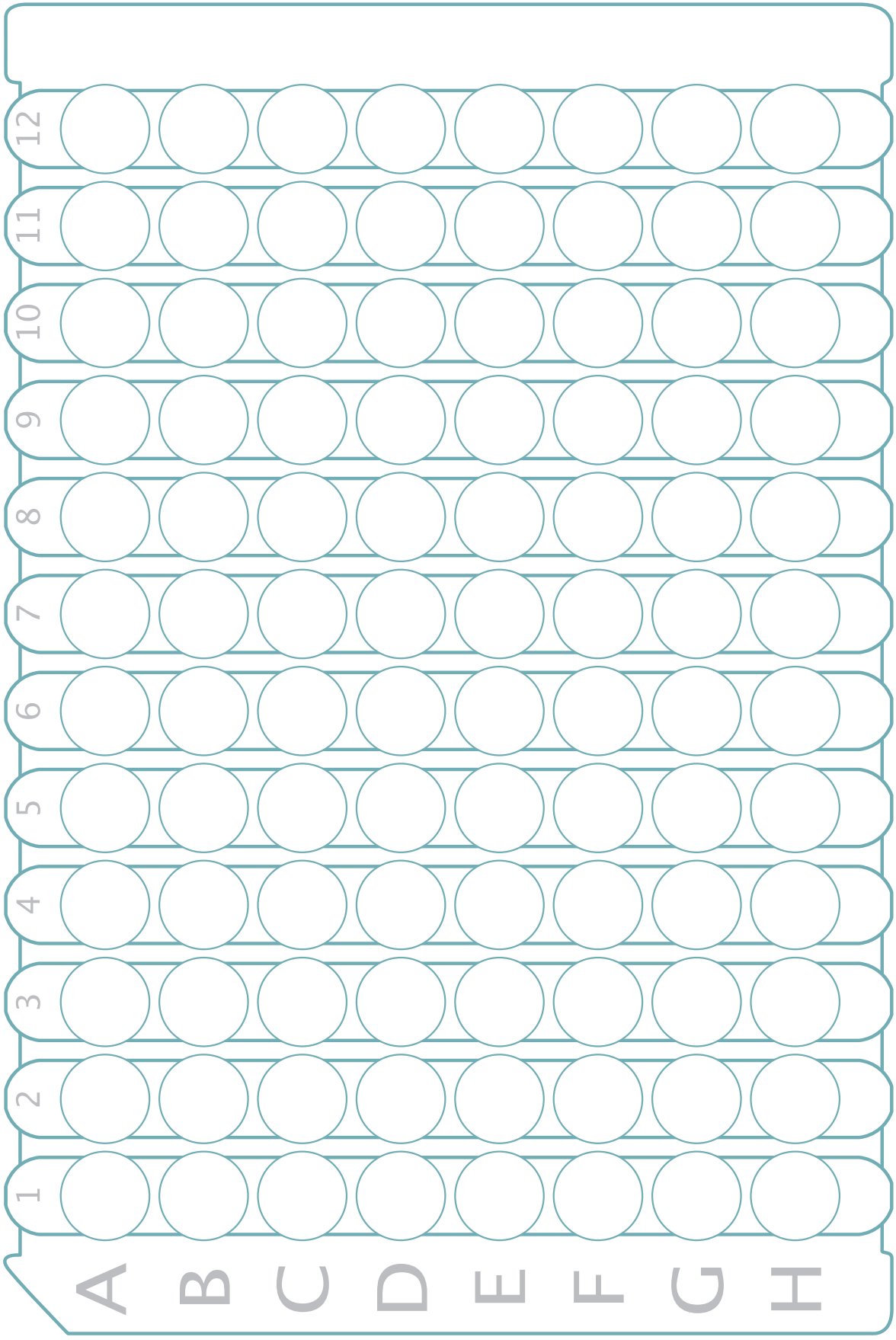
porcine TNF-α
rat TNF-α

REFERENCES

1. Beutler, B. and A. Cerami (1988) Annu. Rev. Biochem. **57**:505.
2. Vilcek, J. and T.H. Lee (1991) J. Biol. Chem. **266**:7313.
3. Dembic, Z. *et al.* (1990) Cytokine **2**:231.
4. Schall, T.J. *et al.* (1990) Cell **61**:361.
5. Loetscher, H. *et al.* (1990) Cell **61**:351.
6. Seckinger, P. *et al.* (1989) J. Biol. Chem. **264**:11966.
7. Olsson, I. *et al.* (1989) Eur. J. Haematol. **42**:270.
8. Engelmann, H. *et al.* (1990) J. Biol. Chem. **265**:1531.
9. Aderka, D. *et al.* (1992) Lymphokine and Cyokine Res. **3**:157.
10. Chouaib, S. *et al.* (1991) Immunology Today **12**:141.
11. Austgulen, R. *et al.* (1992) J. Reproductive Immunol. **22**:105.
12. Van Zee, K. *et al.* (1992) Proc. Natl. Acad. Sci. USA **89**:4845.
13. Spinas, G. *et al.* (1992) J. Clin. Invest. **90**:533.
14. Girardin, E. *et al.* (1992) Immunology **76**:20.
15. Kalinkovich, A. *et al.* (1992) Clin. Exp. Immunol. **89**:351.
16. Andus, T. *et al.* (1992) Hepatology **16**:749.
17. Porteu, F. and C. Nathan (1990) J. Exp. Med. **172**:599.
18. Aderka, D. *et al.* (1992) J. Exp. Med. **175**:323.
19. Tartaglia, L.A. and D.V. Goeddel (1992) Immunology Today **13**:151.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES