Quantikine® ELISA

Human TNF RI/TNFRSF1A Immunoassay

Catalog Number DRT100
SRT100
PDRT100

For the quantitative determination of human Tumor Necrosis Factor Receptor I (TNF RI) 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.

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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 of the factors 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 (Type A, Type a, 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 (Type B, Type b, 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.

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 receptors 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 (12, 13), meningiococcemia (14), and HIV infection (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 the shedding of the two types of soluble receptors is 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 RI/TNFRSF1A Immunoassay is a 4.5 hour solid phase ELISA designed to measure TNF RI in cell culture supernates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant human TNF RI, as well as antibodies raised against this polypeptide. This immunoassay has been shown to accurately quantitate the recombinant TNF RI. Results obtained on samples containing human TNF RI 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 of human TNF RI. Since the measurement of human TNF RI by this immunoassay is relatively 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 human TNF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human TNF RI has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any TNF RI present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human TNF RI 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 RI 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.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell culture supernate samples require large dilutions, perform an intermediate dilution with culture media and the final dilution with the appropriate calibrator diluent.
- 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 SDS 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 # DRT100	CATALOG # SRT100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human TNF RI Microplate	890117	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human TNF RI.	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 RI Conjugate	890118	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human TNF RI conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human TNF RI Standard	890119	1 vial	6 vials	Recombinant human TNF RI in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent HD1-7	895160	1 vial	6 vials	6 mL/vial of a buffered protein base with preservatives.	
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.

DRT100 contains sufficient materials to run an ELISA on one 96 well plate.
SRT100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDRT100). 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.
- Test tubes for dilution of standards and samples.
- Human TNF RI Controls (optional; 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.

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

Caution: *Human serum used in the preparation of cell culture media may contain high levels of TNF RI. Because of the low species cross-reactivity of this kit, human TNF RI levels in culture media containing 10% bovine or fetal bovine serum can be assayed without interference.*

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

Serum and plasma samples require at least a 10-fold dilution in Calibrator Diluent RD6O. 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 in Calibrator Diluent RD5-5. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5-5.

REAGENT PREPARATION

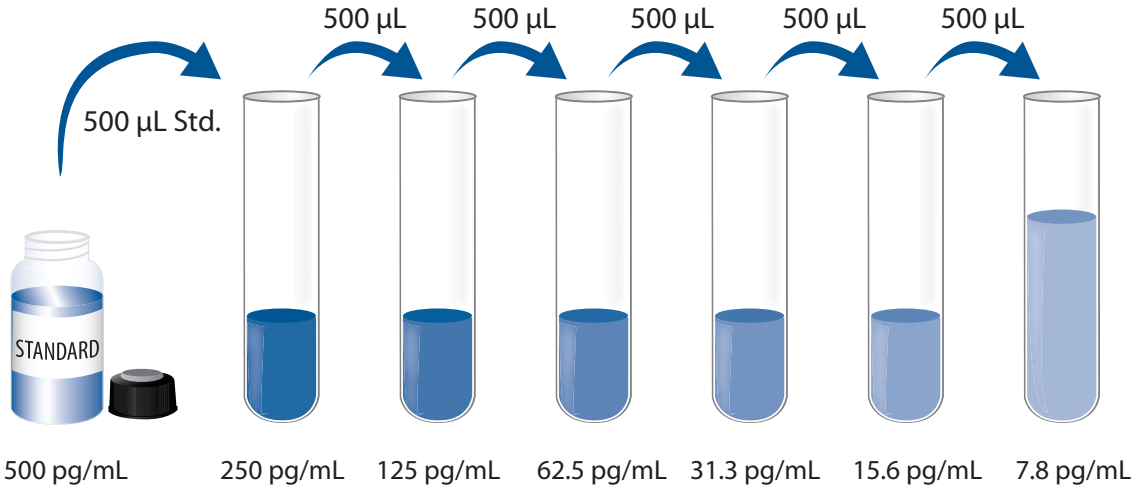
Bring all reagents to room temperature before use.

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 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. 200 µL of the resultant mixture is required per well.

Human TNF RI Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human TNF RI Standard with Calibrator Diluent RD5-5 (*for cell culture supernate or 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.

Pipette 500 µL of Calibrator Diluent RD5-5 (*for cell culture supernate or 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-RI 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, controls, and samples 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 µL of Assay Diluent HD1-7 to each well.
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 towels.
6. Add 200 µL of Human TNF RI 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 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. If the color in the well 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.

*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.).

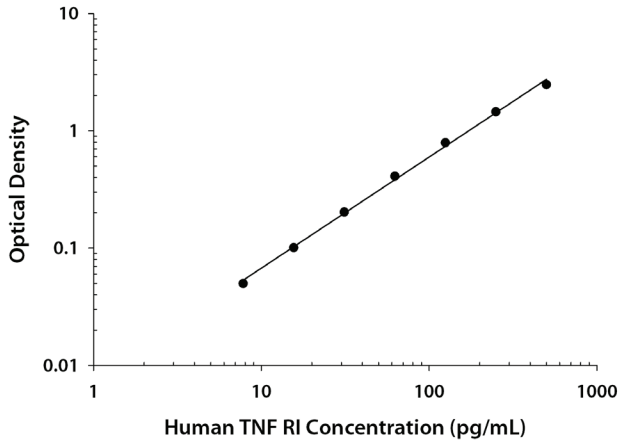
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human TNF RI concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

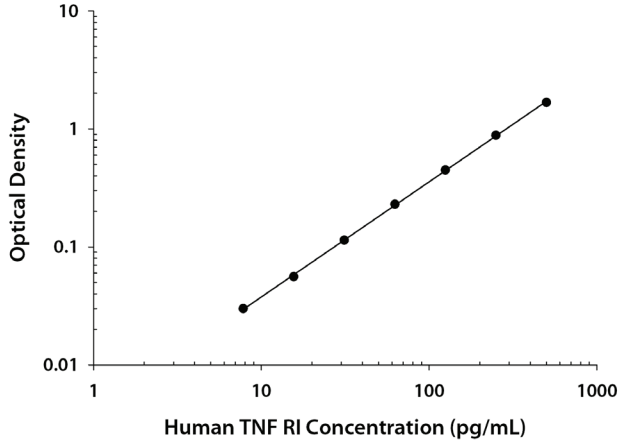
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.033 0.034	0.033	—
7.8	0.082 0.084	0.083	0.050
15.6	0.132 0.137	0.134	0.101
31.3	0.234 0.238	0.236	0.203
62.5	0.433 0.453	0.443	0.410
125	0.804 0.847	0.826	0.793
250	1.340 1.630	1.485	1.452
500	2.471 2.545	2.508	2.475

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.026 0.026	0.026	—
7.8	0.054 0.057	0.056	0.030
15.6	0.080 0.085	0.082	0.056
31.3	0.138 0.143	0.140	0.114
62.5	0.245 0.266	0.256	0.230
125	0.462 0.486	0.474	0.448
250	0.880 0.935	0.908	0.882
500	1.698 1.701	1.700	1.674

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)	41.5	122	231	54.0	251	350
Standard deviation	2.14	5.38	11.0	2.7	9.7	23.3
CV (%)	5.2	4.4	4.8	5.0	3.9	6.7

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)	69.0	198	355	54.8	252	356
Standard deviation	3.24	7.17	17.8	4.8	9.3	20.6
CV (%)	4.7	3.6	5.0	8.8	3.7	5.8

RECOVERY

The recovery of human TNF RI spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	85	80-92%
Serum (n=8)	90	77-103%
EDTA plasma (n=8)	86	70-97%
Heparin plasma (n=8)	93	79-103%
Urine (n=8)	85	71-109%

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of human TNF RI ranged from 0.43-1.20 pg/mL. The mean MDD was 0.77 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 the linearity of the assay, samples containing and/or spiked with high concentrations of human TNF RI were diluted with the appropriate calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=8)	Serum* (n=13)	EDTA plasma* (n=13)	Heparin plasma* (n=13)	Citrate plasma* (n=8)	Urine* (n=13)
1:2	Average % of Expected	106	100	98	97	90	106
	Range (%)	99-115	93-107	92-108	82-112	85-98	97-114
1:4	Average % of Expected	101	101	100	96	89	105
	Range (%)	93-107	94-109	90-110	82-106	81-98	94-122
1:8	Average % of Expected	99	99	100	93	91	104
	Range (%)	85-119	88-106	90-115	81-101	82-99	97-120
1:16	Average % of Expected	99	98	99	88	87	88
	Range (%)	77-114	85-106	86-121	82-97	81-94	81-118

*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 RI produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human TNF RI 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)	1198	749-1966	256
EDTA plasma (n=40)	914	484-1407	208
Heparin plasma (n=40)	1015	512-1739	245
Citrate plasma (n=40)	856	488-1598	210
Urine* (n=33)	1029	173-4030	832

*Values are actual and 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. Cells were stimulated with 10 µg/mL PHA, 10 µg/mL PHA + 10 ng/mL recombinant human (rh) IL-2, 50 ng/mL PMA, or 50 ng/mL LPS. Aliquots of the cell culture supernates were removed on days 1, 3, and 5 and assayed for levels of human TNF RI.

Stimulant	Day 1 (pg/mL)	Day 3 (pg/mL)	Day 5 (pg/mL)
PHA	24	76	141
PHA + rhIL-2	26	71	143
PMA	16	27	67
LPS	17	36	49

SPECIFICITY

This assay recognizes natural and recombinant human TNF RI.

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

Recombinant human:

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

Recombinant mouse:

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

Other recombinants:

amphibian TGF-β5
chicken TGF-β3

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
human TGF-β1
porcine TGF-β1
porcine TGF-β1.2
porcine TGF-β2

Although recombinant human TNF-α, recombinant mouse TNF-α, recombinant rat TNF-α, and recombinant porcine TNF-α did not show any significant cross-reactivity with human TNF RI in this immunoassay, these factors did show a low level of interference. When these factors were added to a mid level human TNF RI control at a concentration of 5.0 ng/mL, the observed value obtained in the immunoassay was decreased by 10%.

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.