Quantikine® ELISA

Mouse Pentraxin 3/TSG-14 Immunoassay

Catalog Number MPTX30

For the quantitative determination of mouse Pentraxin 3 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

Pentraxin 3 (PTX3), also known as Tumor necrosis factor-Stimulated Gene 14 (TSG-14), is a long pentraxin belonging to the pentraxin superfamily. (1-5). The secreted mouse PTX3 is a 45 kDa glycoprotein with a 162 amino acid (aa) N-terminal extension and a 202 aa C-terminal pentraxin domain. The pentraxin domain is homologous to the pentraxin domains found in classical short pentraxins (CRP and SAP) that are produced in the liver (1-4, 6). Pentraxins are multifunctional pattern-recognition proteins that have a characteristic pentagonal discoid arrangement of five non-covalently bound subunits (7, 8). PTX3 also forms decamers and higher-order multimers through the use of interchain disulfide bonds (8). PTX3 is induced in response to proinflammatory stimuli and Toll-like receptor (TLR) engagement. It is secreted by a variety of cell types including macrophages, myeloid derived dendritic cells, ovarian granulosa cells, endothelial cells, fibroblasts, adipocytes, renal mesangial cells, synovial cells, smooth muscle cells, alveolar epithelium, and glial cells (6, 10-18). PTX3 is an acute phase protein in mouse and man. Plasma PTX3 levels increase rapidly during inflammatory and infectious conditions (9-12, 19, 20).

PTX3 binds with high affinity to different soluble ligands and plays an important role in several physiological conditions, ranging from innate immunity to female fertility. Through its interaction with TSG-6, an extracellular matrix hyaluronan (HA)-binding protein, PTX3 is involved in the assembly of the hyaluronan (HA)-rich extracellular matrix of the cumulus oophorus, which is essential for female fertility (4, 14, 21). PTX3 has a dual role in the regulation of the innate immune response. Via its C-terminal pentraxin domain, immobilized and fluid-phase PTX3 will bind complement component C1q to both induce and inhibit, respectively, classical complement activation. While PTX3 enhances C1q binding on apoptotic cells, and facilitates the complement-mediated clearance of these cells, it can also bind apoptotic cells and inhibit their uptake by dendritic cells, preventing the onset of an autoimmune response (22-28). PTX3 interacts with selected viral, fungal and bacterial components, providing protection from infection (29-32). A role for PTX3 as an opsonin has recently been demonstrated, implying the existence of a binding site on macrophages and dendritic cells (1, 2). PTX3 also binds the outer membrane protein A (OmpA) of *Enterobacteriaceae* (*e.g.* KpOmpA from *Klebsiella pneumoniae*), helping to amplify an ongoing inflammatory response (31). Finally, PTX3 may play a role in vascularization. The N-terminal extension of PTX3 has been shown to bind FGF basic and inhibit FGF basic-dependent angiogenesis (33-35).

The Quantikine® Mouse Pentraxin 3/TSG-14 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse Pentraxin 3 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse Pentraxin 3 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse Pentraxin 3 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 natural mouse Pentraxin 3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Pentraxin 3 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Pentraxin 3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Pentraxin 3 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 Pentraxin 3 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, further dilute the samples with 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.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse Pentraxin 3 Microplate	893255	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse Pentraxin 3.	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 Pentraxin 3 Conjugate	893256	12 mL of a polyclonal antibody specific for mouse Pentraxin 3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse Pentraxin 3 Standard	893257	Recombinant mouse Pentraxin 3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse Pentraxin 3 Control	893258	Recombinant mouse Pentraxin 3 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-41	895514	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

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. 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: *Citrate plasma has not been validated for use in this assay.*

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution into Calibrator Diluent RD5-26 (diluted 1:4)*. A suggested 4-fold dilution is 30 µL of sample + 90 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

Cell culture supernate samples may require dilution. The dilution method is dependent upon sample values.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Pentraxin 3 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

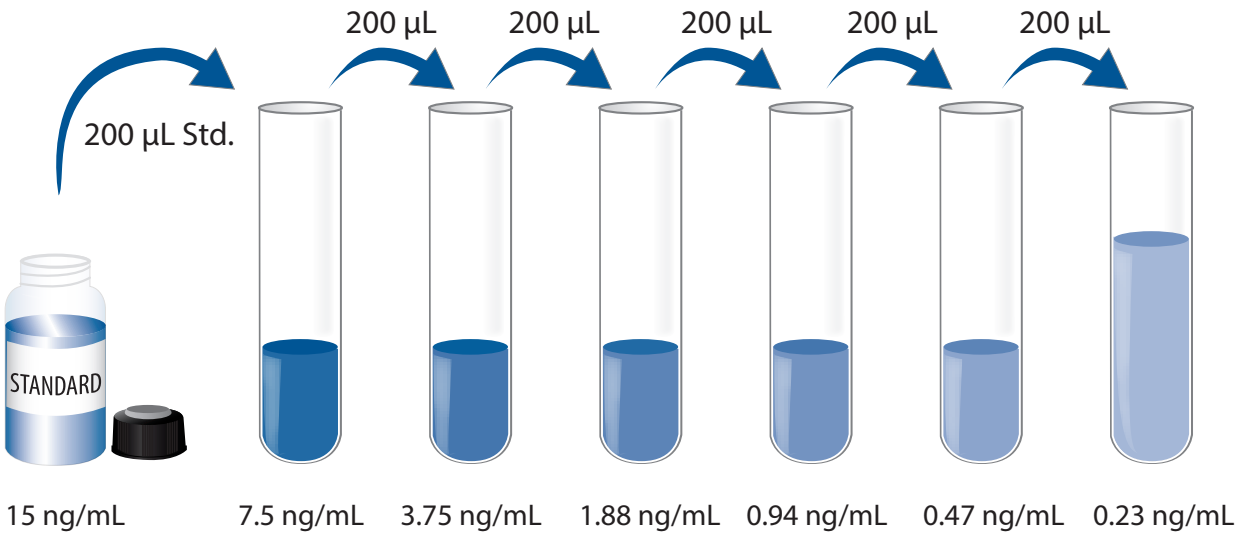
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

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 Pentraxin 3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Pentraxin 3 Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 15 ng/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse Pentraxin 3 Standard (15 ng/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-41 to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 Pentraxin 3 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

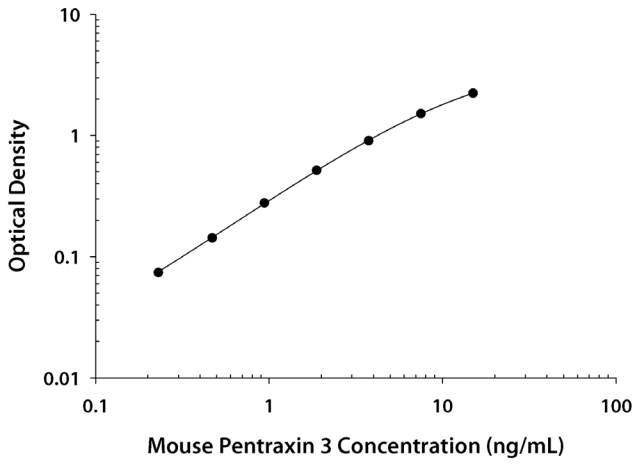
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse Pentraxin 3 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D.	Average	Corrected
0	0.030 0.031	0.031	—
0.23	0.104 0.105	0.105	0.074
0.47	0.171 0.176	0.174	0.143
0.94	0.305 0.313	0.309	0.278
1.88	0.538 0.556	0.547	0.516
3.75	0.921 0.955	0.938	0.907
7.5	1.516 1.574	1.545	1.514
15	2.226 2.299	2.263	2.232

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 separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	33	34	34
Mean (ng/mL)	0.54	2.09	6.86	0.63	2.28	7.18
Standard deviation	0.01	0.05	0.20	0.07	0.16	0.41
CV (%)	1.9	2.4	2.9	11.1	7.0	5.7

RECOVERY

The recovery of mouse Pentraxin 3 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	107	98-119%
Serum* (n=4)	102	90-117%
EDTA plasma* (n=4)	98	90-110%
Heparin plasma* (n=4)	95	81-100%

*Samples were spiked and then diluted 10-fold prior to assay.

SENSITIVITY

Forty-four assays were evaluated and the minimum detectable dose (MDD) of mouse Pentraxin 3 ranged from 0.004-0.020 ng/mL. The mean MDD was 0.012 ng/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 highly purified NS0-derived recombinant mouse Pentraxin 3 produced at R&D Systems®.

LINEARITY

To assess the linearity of the assay, samples containing and/or spike with high concentrations of mouse Pentraxin 3 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=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	94	101	102	104
	Range (%)	90-97	96-104	97-107	101-107
1:4	Average % of Expected	93	105	105	104
	Range (%)	88-100	101-108	102-109	102-106
1:8	Average % of Expected	91	107	103	103
	Range (%)	86-98	106-109	101-107	98-109
1:16	Average % of Expected	90	107	105	105
	Range (%)	83-98	104-115	101-115	99-112

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

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse Pentraxin 3 in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=20)	9.5	5.3-17	2.9
EDTA plasma (n=20)	8.1	4.7-12	2.3
Heparin plasma (n=20)	7.7	4.0-12	2.6

Cell Culture Supernates - 3T3-L1 mouse embryonic fibroblast adipose-like cells were grown in DMEM supplemented with 10% fetal bovine serum until 80% confluent then cultured for 3 days. The cell culture supernate was assayed for mouse Pentraxin 3 and measured 28 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse Pentraxin 3.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse Pentraxin 3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
CRP
SAP
TSG-6

Recombinant rat:
CRP
SAP

Recombinant human:
Pentraxin 3
TSG-6

REFERENCES

1. Bottazzi, B. *et al.* (2006) J. Leukoc. Biol. **79**:909.
2. Bottazzi, B. *et al.* (2005) Curr. Opin. Immunol. **18**:10.
3. Garlanda, C. *et al.* (2005) Annu. Rev. Immunol. **23**:337.
4. Wisniewski, H. and J. Vilcek (2004) Cytokine Growth Factor Rev. **15**:129.
5. Lee, G.W. *et al.* (1993) J. Immunol. **150**:1804.
6. Introna, M. *et al.* (1996) Blood **87**:1862.
7. Emsley, J. *et al.* (1994) Nature **367**:338.
8. Bottazzi, B. *et al.* (1997) J. Biol. Chem. **272**:32817.
9. Goodman, A.R. *et al.* (2000) J. Leukoc. Biol. **67**:387.
10. Breviario, F. *et al.* (1992) J. Biol. Chem. **267**:22190.
11. Abderrahim-Ferkoune, A. *et al.* (2003) J. Lipid Res. **44**:994.
12. Bussolati, B. *et al.* (2003) J. Immunol. **170**:1466.
13. Doni, A. *et al.* (2003) Eur. J. Immunol. **33**:2886.
14. Salustri, A. *et al.* (2004) Development **131**:1577.
15. Luchetti, M.M. *et al.* (2000) Clin. Exp. Immunol. **119**:196.
16. Klouche, M. *et al.* (2004) Atherosclerosis **175**:221.
17. dos Santos, C.C. *et al.* (2004) Physiol. Genomics **19**:331.
18. Polentarutti, N. *et al.* (2000) J. Neuroimmunol. **106**:87.
19. Lee, G.W. *et al.* (1994) J. Immunol. **153**:3700.
20. Doni, A. *et al.* (2006) J. Leukoc. Biol. **79**:797.
21. Varani, S. *et al.* (2002) Mol. Endocrinol. **16**:1154.
22. Nauta, A.J. *et al.* (2003) Eur. J. Immunol. **33**:465.
23. Rovere, P. *et al.* (2000) Blood **96**:4300.
24. Baruah, P. *et al.* (2006) Blood **107**:151.
25. Diniz, S.N. *et al.* (2004) J. Leukoc. Biol. **75**:649.
26. Roumenina, L.T. *et al.* (2006) Biochemistry **45**:4093.
27. Baruah, P. *et al.* (2006) J. Leukoc. Biol. **80**:87.
28. Inforzato, A. *et al.* (2006) Biochemistry **45**:11540.
29. Garlanda, C. *et al.* (2002) Nature **420**:182.
30. Dias, A.A.M. *et al.* (2001) J. Leukoc. Biol. **69**:928.
31. Jeannin, P. *et al.* (2005) Immunity **22**:551.
32. Bozza, S. *et al.* (2006) Blood **108**:3387.
33. Rusnati, M. *et al.* (2004) Blood **104**:92.
34. Camozzi, M. *et al.* (2005) Arterioscler. Thromb. Vasc. Biol. **25**:1837.
35. Camozzi, M. *et al.* (2006) J. Biol. Chem. **281**:22605.