Quantikine® ELISA

Mouse Pentraxin 2/SAP Immunoassay

Catalog Number MPTX20

For the quantitative determination of mouse Pentraxin 2 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

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INTRODUCTION

Pentraxin 2 (PTX2), also known as Serum Amyloid P Component (SAP), is a monomeric 25 kDa secreted serum glycoprotein that is a member of the pentraxin family whose members have a characteristic cyclic pentameric structure (1-3). PTX2 is a pattern-recognition molecule that is active in the humoral arm of innate immunity, and is also a component of normal basement membranes and amyloid deposits (1-6). PTX2 and C-reactive protein (CRP) belong to the classical (short) pentraxin subfamily and are major acute-phase plasma proteins in mice and humans (3, 5-7). PTX2 is secreted by hepatocytes in response to inflammatory cytokines (6-8). Mouse PTX2 shares 79% and 69% amino acid (aa) sequence identity with rat and human PTX2, respectively. It also shares 46% aa sequence identity with mouse CRP (1).

PTX2 shows specific calcium-dependent lectin-like binding to various microbial components and self-antigens including DNA and chromatin derived from damaged cells (1, 2, 6, 9). Upon binding to its ligands, PTX2 is involved in the activation of the classical complement cascade (1). PTX2 binds to and interacts with Fcγ receptors to promote phagocytosis of microbial organisms and apoptotic cells (4, 10). Its signaling through Fcγ receptors also reduces the differentiation of pro-fibrotic M2 macrophages from monocytes, thus inhibiting fibrosis (11-13). PTX2 may also downregulate acute inflammation accompanying experimental autoimmune encephalomyelitis (EAE) by regulating the T-cell activity (14). In addition to its role in innate immunity, PTX2 is a universal component of amyloid deposits, which are implicated in a diverse range of human diseases including Alzheimer's, prion diseases, type 2 diabetes, and various systemic amyloidoses (1, 3, 15, 16). PTX2 stabilizes amyloid fibrils and protects them from proteolytic degradation (1, 3). Deletion of PTX2 from certain mouse strains can delay the deposition of amyloids and increase lupus-like autoimmunity to chromatin (9, 17).

Mouse strains vary in baseline plasma PTX2 concentration, but may show similar PTX2 concentrations following induction (6, 8). Strains having low baseline PTX2 expression therefore generally show the most change, and are termed high responders. Reported response is high (up to 20-fold) for C57BL/6, B6D2F1, AKR, DBA/1, and CBA/J, intermediate for BALB/c, B6AF1, and C3H/HeN, and low (down to 2-fold) for DBA/2, A/J, and C3H/HeJ strains (8).

The Quantikine® Mouse Pentraxin 2/SAP Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse Pentraxin 2 in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse Pentraxin 2 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant factor accurately. Results obtained using natural mouse Pentraxin 2 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 Pentraxin 2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse Pentraxin 2 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any Pentraxin 2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse Pentraxin 2 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 2 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.

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.

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 2 Microplate	894077	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse Pentraxin 2.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse Pentraxin 2 Standard	894079	2 vials of recombinant mouse Pentraxin 2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse Pentraxin 2 Control	894080	2 vials of recombinant mouse Pentraxin 2 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse Pentraxin 2 Conjugate	894078	12 mL of a polyclonal antibody specific for mouse Pentraxin 2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD5-60	895978	2 vials (21 mL/vial) of a buffered protein base with preservatives. *Use diluted 1:5 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.

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

Cell culture supernate samples require a 5-fold dilution. A suggested 5-fold dilution can be achieved by adding 30 µL of sample to 120 µL of Calibrator Diluent RD5-60 (diluted 1:5)*.

ACID TREATMENT PROCEDURE

Serum and plasma sample require an acid pretreatment with the Stop Solution provided in this kit. Discard any unused acid treated sample.

NOTE: ***Do not acid treat the standards, control, or cell culture supernate samples.***

1. To 20 µL of serum/plasma, add 20 µL of Stop Solution.
2. Mix well.
3. Incubate for 10 minutes at room temperature.
4. Dilute the acidified sample with the indicated volume of Calibrator Diluent RD5-60 (diluted 1:5)*.

Mouse Non-Swiss Albino (NSA) acid treated serum and plasma samples require a 600-fold dilution. A 600-fold dilution can be achieved by adding 10 µL of acid treated sample to 190 µL of Calibrator Diluent RD5-60 (diluted 1:5)*. Complete the 600-fold dilution by adding 10 µL of this mixture to 290 µL of Calibrator Diluent RD5-60 (diluted 1:5)*. The final sample dilution is 1200-fold.

Mouse Balb/c acid treated serum and plasma samples require a 400-fold dilution. An 400-fold dilution can be achieved by adding 10 µL of acid treated sample to 190 µL of Calibrator Diluent RD5-60 (diluted 1:5)*. Complete the 400-fold dilution by adding 10 µL of this mixture to 190 µL of Calibrator Diluent RD5-60 (diluted 1:5)*. The final sample dilution is 800-fold.

Mouse C57 acid treated serum and plasma samples require a 100-fold dilution. A 100-fold dilution can be achieved by adding 10 µL of acid treated sample to 990 µL of Calibrator Diluent RD5-60 (diluted 1:5)*. The final sample dilution is 200-fold.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Mouse Pentraxin 2 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

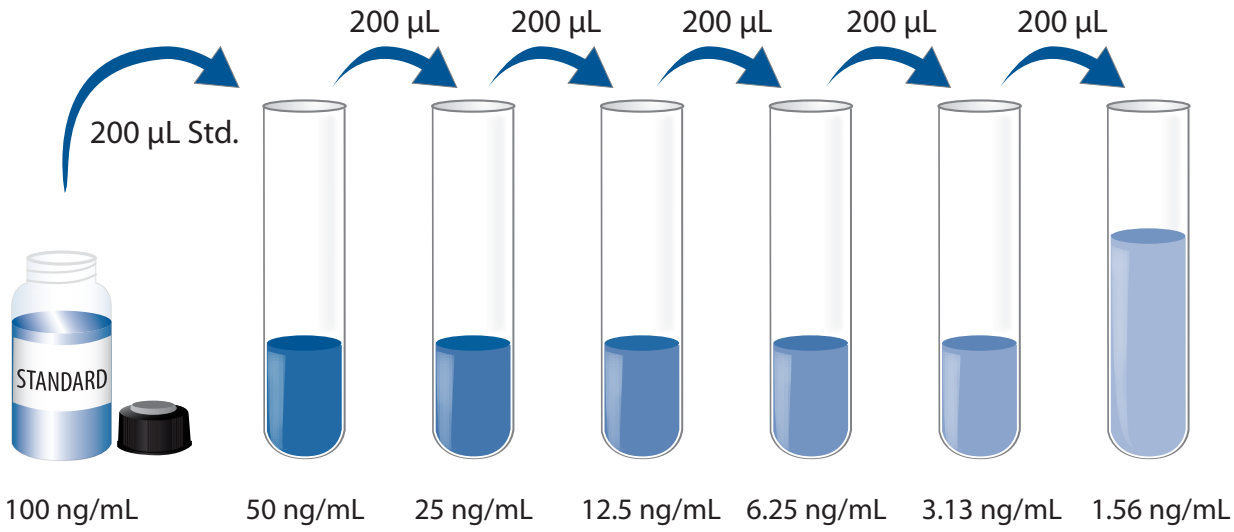
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5-60 (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5-60 to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5-60 (diluted 1:5).

Mouse Pentraxin 2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse Pentraxin 2 Standard with Calibrator Diluent RD5-60 (diluted 1:5). This reconstitution produces a stock solution of 100 ng/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-60 (diluted 1:5) 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 2 Standard (100 ng/mL) serves as the high standard. Calibrator Diluent RD5-60 (diluted 1:5) 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, working standards, 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 Calibrator Diluent RD5-60 (diluted 1:5) to each well.
4. Add 50 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for **3 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 2 Conjugate to each well. Cover with a new adhesive strip. Incubate for **1 hour** 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.

*Serum and plasma samples require acid treatment and dilution. Cell culture supernate samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

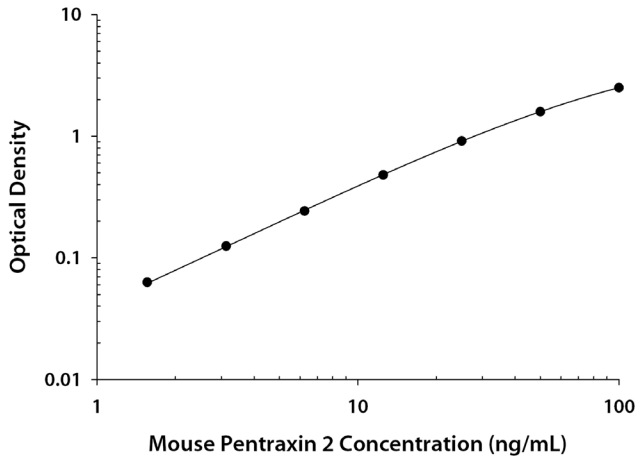
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse Pentraxin 2 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.

Since 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	0.056 0.059	0.058	—
1.56	0.116 0.126	0.121	0.063
3.13	0.180 0.185	0.183	0.125
6.25	0.298 0.304	0.301	0.243
12.5	0.536 0.539	0.538	0.480
25	0.971 0.973	0.972	0.914
50	1.644 1.647	1.646	1.588
100	2.540 2.587	2.564	2.506

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/mL)	4.11	16.0	33.6	3.95	15.7	34.5
Standard deviation	0.22	0.57	1.33	0.33	0.76	1.59
CV (%)	5.4	3.6	4.0	8.4	4.8	4.6

RECOVERY

The recovery of mouse Pentraxin 2 spiked into cell culture samples was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples* (n=6)	97	81-112%

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

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of mouse Pentraxin 2 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were acid treated and/or diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	100	98	92	92
	Range (%)	91-117	94-104	87-96	88-98
1:4	Average % of Expected	98	92	88	88
	Range (%)	95-102	86-99	86-90	84-99
1:8	Average % of Expected	101	91	87	89
	Range (%)	101-102	82-104	82-95	85-99
1:16	Average % of Expected	107	86	86	87
	Range (%)	101-114	80-97	82-90	80-101

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SENSITIVITY

Seventy assays were evaluated and the minimum detectable dose (MDD) of mouse Pentraxin 2 ranged from 0.050-0.368 ng/mL. The mean MDD was 0.159 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-expressed recombinant mouse Pentraxin 2 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse Pentraxin 2 in this assay.

NSA Mouse Strain	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum (n=20)	25.0	7.93-78.0	16.4
EDTA plasma (n=20)	23.3	10.1-47.8	11.8
Heparin plasma (n=20)	24.8	4.12-52.3	14.8

Balb/c Strain	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum & plasma (n=6)	21.4	15.8-29.8	5.4

C57 Strain	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum & plasma (n=6)	7.1	2.9-12.9	3.4

Cell Culture Supernates - Organs from mice were removed, rinsed in 1X PBS, and kept on ice in 1X PBS. Organs were then homogenized and seeded into media containing 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 for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of mouse Pentraxin 2.

Tissue Type	(ng/mL)
Kidney	222
Lung	24.4

SPECIFICITY

This assay recognizes natural and recombinant mouse Pentraxin 2.

The factors listed below were prepared at 1.0 µg/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 1.0 µg/mL in a mid-range recombinant mouse PTX2/SAP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	Recombinant human:
CRP	CRP
Fcγ RI/CD64	Fcγ RIIA/CD32a
Fcγ RIIB/CD32b	Fcγ RIIB/C (CD32b/c)
Fcγ RIII/CD16	Fcγ RIIIA/CD16a
TSG	Fcγ RIIIB/CD16b
TSG-6	TSG-6
TSG-14	TSG-14

Recombinant human Pentraxin 2 cross-reacts 0.4% in this assay.

Recombinant rat Pentraxin 2 cross-reacts 4.0% in this assay.

Recombinant rat CRP cross-reacts approximately 0.2% in this assay.

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