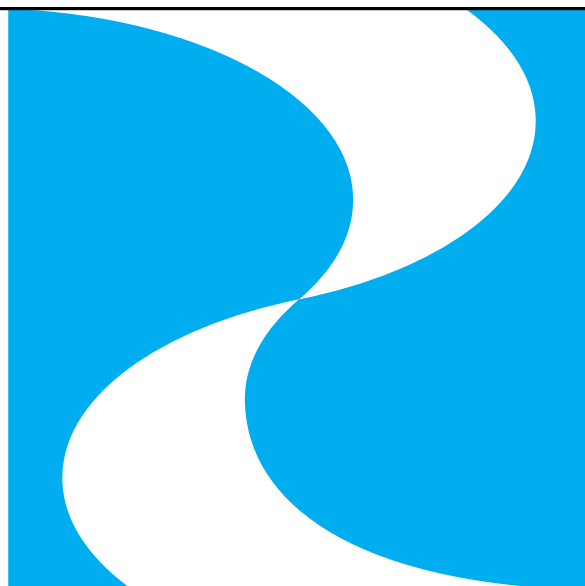


Human Pentraxin3 / TSG-14 ELISA System

Immunosorbent assay for the quantitative measurement of human Pentraxin3 (PTX3) in plasma and serum-free cell culture supernatant.



*FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.*

Product code: PP-PD03-E0

Storage: Store all reagents at 2~8°C

Expiry: The expiry date is stamped on the package.

Distributed by
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Manufactured by



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1. Handling

(1) Safety warnings and precautions

This kit is for RESEARCH USE ONLY. Not recommended or intended for diagnosis of disease in humans or animals. For safety reasons, all chemicals included in this kit should be considered as potentially hazardous. Wear suitable protective clothing such as laboratory coat, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes, wash immediately with copious amounts of water.

(2) Storage and stability

All reagents should be stored in refrigerator at 2~8°C upon receipt.

Do not freeze reagents.

Please refer to the expiry date on the box.

Do not use kit beyond the stated expiry date.

2. Components

(1) Materials provided

This kit contains the following assay components, sufficient for 96 wells.

- Anti-human PTX3 antibody precoated strip well plate, 1 (96 well)
- HRP conjugated anti-PTX3 antibody reagent, 1 vial (12 mL) containing preservative
- Human PTX3 standards 0, 0.5, 1.0, 2.5, 5.0, 10, 20 ng/mL, 7 vials (0.1 mL), containing 0.1%(w/v) sodium azide
- 10X Wash buffer concentrate, 1 vial (100 mL)
- Dilution buffer, 1 vial (15 mL) containing preservative
- TMB solution, 1 vial (12 mL) containing $\leq 30\%$ Methanol, $\leq 10\%$ Acetone, $< 3\%$ DMSO, $\leq 0.1\%$ 3-3'-5-5' Tetramethyl-Benzidine and $\leq 0.03\%$ Hydrogen Peroxide
- Stop solution, 1 vial (12 mL) containing $< 10\%$ sulfuric acid and $< 10\%$ hydrochloric acid
- Adhesive plate covers, 3 sheets
- Instruction manual, 1

(2) Additional materials required

- Pipettes or pipetting equipment with disposable tips (20 μ L, 100 μ L, 1000 μ L)
- Distilled or deionized water for wash buffer
- Measuring cylinder to prepare wash buffer
- Plate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 620 nm.
- Laboratory glassware.
- Horizontal orbital microplate shaker capable of maintaining a speed of around 400 ~700 rpm
- Optional; Microtiter plate washer for high throughput applications

3. Critical parameters

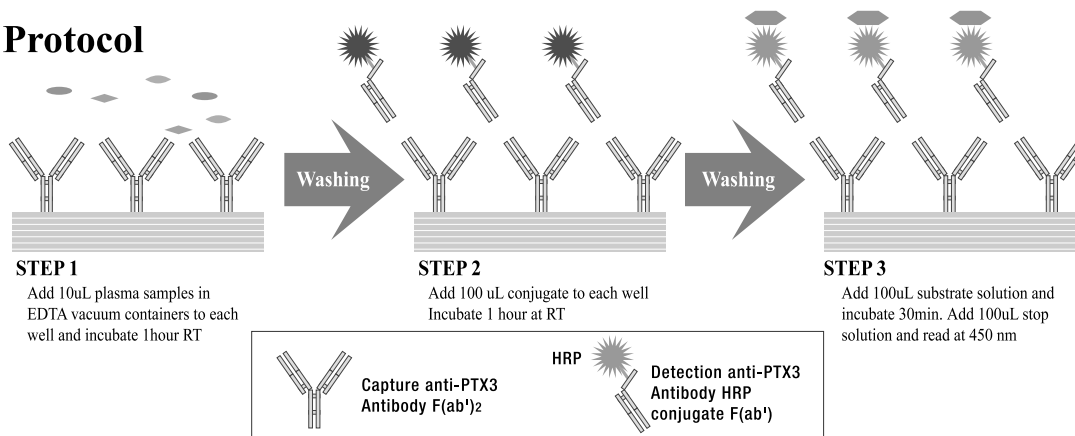
- All specimens and reagents must be warmed to room temperature (20~25°C) prior to use. Samples should be thawed at room temperature. Do not use water baths to thaw samples.
- Do not mix reagents from different kit lots.
- Avoid microbial contamination of reagents. If TMB solution is blue prior to use, do not use.
- Avoid exposure of reagents to excessive heat or light during storage and incubation.
- Many individual components contain preservative. Appropriate protective clothing, glasses and gloves should be worn at all times when handling kit reagents to avoid direct skin contact.
- Do not use glass pipettes to measure the TMB solution.
- TMB solution provided with this kit contains $\leq 30\%$ Methanol, $\leq 10\%$ Acetone, $< 3\%$ DMSO, $\leq 0.1\%$ 3-3'-5-5' Tetramethyl-Benzidine and $\leq 0.03\%$ Hydrogen Peroxide. The stop solution provided with this kit contains $< 10\%$ sulfuric acid and $< 10\%$ hydrochloric acid solution. Wear eyes, hand, face and clothing protection at all times, when using this reagent.
- PTX3 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while performing assay.

4. Introduction

Human Pentraxin3 / TSG-14 ELISA System is a solid-phase sandwich immunoassay capable of measuring human PTX3 levels in plasma and serum free cell culture supernatant.

Pentraxins are a superfamily of conserved proteins characterized by the pentraxin domain (1-6). C-reactive protein (CRP) and serum amyloid protein (SAP) are recognized as classical short pentraxins, whereas pentraxin3 (PTX3) belongs to the long pentraxins. CRP and SAP are induced in the liver in response to IL-6. In contrast, PTX3 is produced by a variety of tissues and cells, such as vascular endothelial cells, macrophages, and neutrophils (7-11), predominantly in response to proinflammatory signals (bacterial products, IL-1, and TNF) (12-22). PTX3 has also been observed in human atherosclerotic lesions of autopsy samples by

Protocol



immunohistochemistry, in association with macrophage and neutrophils infiltration (23). This localized expression pattern of PTX3 is believed to be indicative of primary inflammation stimuli, in contrast to CRP which is induced via secondary signals (24-31).

■ Publications based on human Pentraxin3 / TSG-14 ELISA System

- Inoue K, Sugiyama A, Reid PC, Ito Y, Miyauchi K, Mukai S, Sagara M, Miyamoto K, Satoh H, Kohno I, Kurata T, Ota H, Mantovani A, Hamakubo T, Daida H, Kodama T. Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris. *Arterioscler Thromb Vasc Biol.* 2007 ; 27(1) : 161-7.
- Imamura M, Kawasaki T, Savchenko AS, Ohashi R, Jiang S, Miyamoto K, Ito Y, Iwanari H, Sagara M, Tanaka T, Hamakubo T, Kodama T, Uchiyama M, Naito M. Lipopolysaccharide induced expression of pentraxin 3 in human neutrophils and monocyte-derived macrophages. *Cell Immunol.* 2007 ; 248(2) : 86-94.
- Tong M, Carrero JJ, Qureshi AR, Anderstam B, Heimbürger O, Barany P, Axelsson J, Alvestrand A, Stenvinkel P, Lindholm B, Suliman ME. Plasma pentraxin 3 in patients with chronic kidney disease: associations with renal function, protein-energy wasting, cardiovascular disease, and mortality. *Clin J Am Soc Nephrol.* 2007 ; 2(5) : 889-97.
- Suzuki S, Takeishi Y, Niizeki T, Koyama Y, Kitahara T, Sasaki T, Sagara M, Kubota I. Pentraxin 3, a new marker for vascular inflammation, predicts adverse clinical outcomes in patients with heart failure. *Am Heart J.* 2008 ; 155(1) : 75-81.
- Kotooka N, Inoue T, Fujimatsu D, Morooka T, Hashimoto S, Hikichi Y, Uchida T, Sugiyama A, Node K. Pentraxin3 is a novel marker for stent-induced inflammation and neointimal thickening. *Atherosclerosis.* 2008 ; 197(1) : 368-74.
- Savchenko A, Imamura M, Ohashi R, Jiang S, Kawasaki T, Hasegawa G, Emura I, Iwanari H, Sagara M, Tanaka T, Hamakubo T, Kodama T, Naito M. Expression of pentraxin 3 (PTX3) in human atherosclerotic lesions. *J Pathol.* 2008 ; 215(1) : 48-55.
- Kato S, Ochiai M, Sakurada T, Ohno S, Miyamoto K, Sagara M, Ito M, Takeuchi K, Imaki J, Itoh K, Yakabi K. Increased Expression of Long Pentraxin PTX3 in Inflammatory Bowel Diseases. *Dig Dis Sci.* 2008 ; 53(7) : 1910-6.
- Kotooka N, Inoue T, Aoki S, Anan M, Komoda H, Node K. Prognostic value of pentraxin 3 in patients with chronic heart failure. *Int J Cardiol.* 2008 ; 130(1) : 19-22.

- Yamasaki K, Kurimura M, Kasai T, Sagara M, Kodama T, Inoue K.
Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations.
Clin Chem Lab Med. 2009 ; 47(4) : 471-7. as of June, 2009

5. Preparation

(1) Sample preparation

■ Plasma

Fresh or defrosted human plasma prepared with EDTA. Samples may be stored at $< -20^{\circ}\text{C}$.

After collecting blood, centrifuge for 10 minutes at 3,000 rpm. Assay immediately or aliquot and store samples at $< -20^{\circ}\text{C}$ within 2 hours.

Caution: Serum, heparin plasma and citrate plasma samples can NOT be used with this kit.

■ Cell culture supernatants

Remove particulates by centrifugation and assay immediately or aliquot and store samples at $< -20^{\circ}\text{C}$.

Caution: Using culture supernatant containing serum (FBS) is not recommended, as there is some possibility of assay inhibition by FBS. If you use culture supernatant containing FBS, please verify recovery prior to measuring samples.

(2) Reagent preparation

Before testing, bring all reagents to room temperature ($18\sim 25^{\circ}\text{C}$). All reagents must be carefully mixed before use. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.

■ Human PTX3 Microtiter plate

Determine the appropriate number of strips needed for each assay, leaving the strips to be used in the frame. Remove the unnecessary strips and store them in the foil pouch with the desiccant provided at $2\sim 8^{\circ}\text{C}$, making sure the foil pouch is sealed tightly. After running the assay, retain the plate frame for future assays. In future assays, ensure that the reserved strips are placed securely into the plate frame.

■ Wash buffer concentrate

100 mL of 10X Wash buffer concentrate is supplied in this kit. If performing 96 sample assay, dilute 10-fold to prepare 1000 mL of wash buffer. Do not store diluted wash buffer. If performing partial assay, 2 strips requires about 100 mL of wash buffer. To make 100 mL of wash buffer, add 10 mL of 10X wash buffer concentrate to 90 mL distilled water in a 100 mL cylinder.

6. Assay procedure

1. Bring all reagents and samples to room temperature before use.
2. Prepare the appropriate amount of Wash buffer and microplate strips as directed in the previous sections.
3. Pipet **100 μ L** of Dilution buffer to each well.
4. Add **10 μ L** of each Human PTX3 standard, or sample per well. It is recommended that all samples, controls and standards be assayed in duplicate.
5. Carefully cover the plate with the adhesive plate cover provided. Ensure that all edges and strips are sealed tightly by running a thumb over the edges and down each strip. Incubate for **1 hour** at room temperature (20~25°C) **with mixing** (400~700 rpm).
6. At the end of the incubation period, carefully remove the adhesive plate cover. Aspirate each well and wash, repeating the process four times for a total of five washes.

Wash by filling each well with approximately 400 μ L Wash buffer 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 on to paper towels or other absorbent material.
7. Add **100 μ L** of HRP conjugated anti-PTX3 antibody reagent to each well.
8. Carefully cover with a new adhesive plate cover. Ensure that all edges and strips are sealed tightly by running a thumb over the edges and down each strip. Incubate for **1 hour** at room temperature **with mixing** (400~700 rpm).
9. Aspirate and wash each well 5 times with Washing buffer as in step6.
10. Add **100 μ L** of TMB solution to each well and incubate **30 minutes** at room temperature **in the dark** or protected from light.
11. Add **100 μ L** 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 the color change does not appear uniform, gently tap the plate to ensure **thorough mixing**.
12. Determine the optical density of each well within 30 minutes.
Measure the absorbance on a microplate reader set at 450 nm with a wavelength correction set at 620 nm. Subtract reading at 620 nm from the readings at 450 nm, reading at dual wavelength will correct for optical imperfection in the microplate. If a wavelength correction is not available, read the plate at 450 nm only.

Please Note: When the 620 nm adjustment is omitted, OD values will be higher. The plate must be read **within 30 minutes** after stopping the reaction.

7. Calculation of results

The following algorithms can be used alternatively to calculate the results.

■ Point to point calculation

We recommend a linear ordinate for optical density and a linear abscissa for concentration.

■ 4-parameter-algorithm

We recommend a logarithmic ordinate for optical density and a logarithmic abscissa for concentration.

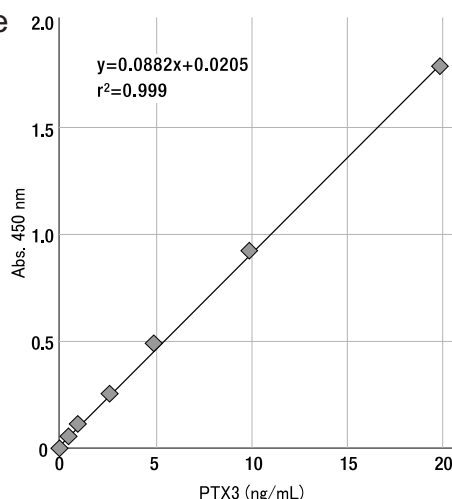
8. Limitations

Plasma samples with PTX3 levels greater than the highest standard value, should be diluted with Dilution buffer, and re-assayed.

Optical density values obtained for duplicates should be within 10% of the mean. Duplicate values that differ from the mean by greater than 10% should be considered unreliable and should be repeated.

9. Assay range

■ Standard curve



		Abs.450nm			Mean (ng/mL)	SD	CV	NET (ng/mL)
		Well 1	Well 2	Well 3				
Human PTX3 standards	0.0	0.011	0.010	0.011	0.011	0.001	5.4%	0.000
	0.5	0.069	0.074	0.070	0.071	0.003	3.7%	0.060
	1.0	0.107	0.108	0.103	0.106	0.003	2.5%	0.095
	2.5	0.258	0.263	0.256	0.259	0.004	1.4%	0.248
	5.0	0.504	0.499	0.486	0.496	0.009	1.9%	0.486
	10	0.937	0.933	0.946	0.939	0.007	0.7%	0.928
	20	1.799	1.750	1.779	1.776	0.025	1.4%	1.765

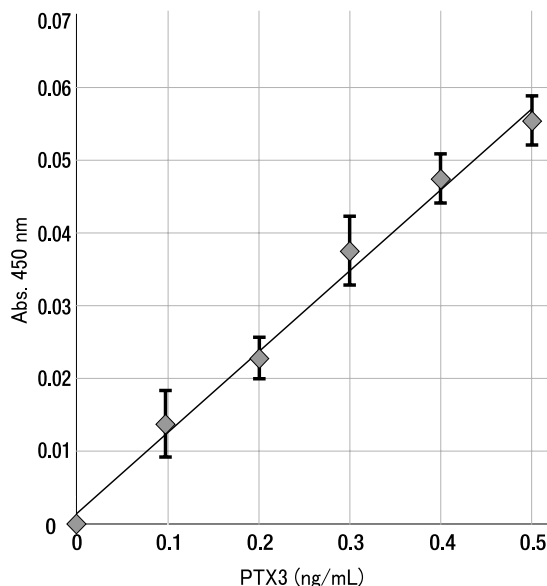
Representative standard curve based on PTX3 calibrators of 0.5~20 ng/mL at 450 nm is shown. This standard curve is provided for demonstration only. A standard curve should be performed each time PTX3 values are measured.

10. Performance characteristics

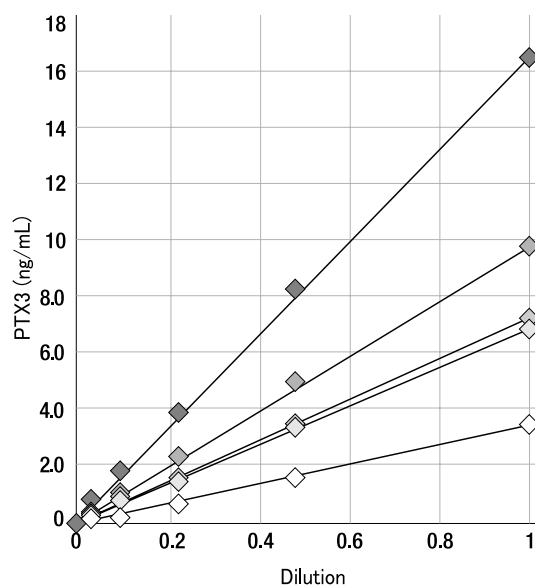
(1) Sensitivity and Linearity of dilution

The lower limit of detection is 0.1 ng/mL. Dilution curves of plasma samples show good linearity.

■ Sensitivity



■ Linearity



(2) Reproducibility

■ Intra-assay

CV : <4.1%

Sample No.	Well 1	Well 2	Well 3	Well 4	Well 5	Mean (ng/mL)	SD	CV
1	15.88	16.10	15.98	15.76	15.84	15.91	0.13	0.8%
2	7.22	7.05	7.30	7.14	7.13	7.17	0.10	1.3%
3	9.85	9.91	9.18	9.76	9.79	9.70	0.30	3.0%
4	0.93	0.94	0.94	0.94	0.97	0.95	0.02	1.6%
5	0.81	0.82	0.89	0.83	0.87	0.85	0.03	4.1%
6	0.64	0.66	0.69	0.66	0.68	0.67	0.02	2.9%

■ Inter-assay

CV : <4.3%

Sample No.	Experiments			Mean (ng/mL)	SD	CV
	1st	2nd	3rd			
1	15.41	16.68	15.76	15.95	0.66	4.1%
2	7.04	7.46	7.13	7.21	0.22	3.1%
3	9.54	10.17	9.38	9.70	0.42	4.3%
4	0.94	0.97	0.93	0.95	0.02	2.2%
5	0.84	0.91	0.87	0.87	0.03	4.0%
6	0.66	0.67	0.64	0.66	0.01	1.8%

(3) Specificity

Cross reactivity with human CRP and SAP is <0.1 ng/mL.

human CRP

human SAP

PTX3 standards	PTX3 Standard	NET	CRP	Apply	NET	PTX3 (ng/mL)	SAP	Apply	NET	PTX3 (ng/mL)
	0 ng/mL	0.000		0 ng/mL	0.000	<0.1 ng/mL		0 ng/mL	0.001	<0.1 ng/mL
	0.5 ng/mL	0.057		10 ng/mL	0.004	<0.1 ng/mL		10 ng/mL	0.005	<0.1 ng/mL
	1.0 ng/mL	0.111		20 ng/mL	0.003	<0.1 ng/mL		20 ng/mL	0.003	<0.1 ng/mL
	2.5 ng/mL	0.270		50 ng/mL	0.007	<0.1 ng/mL		50 ng/mL	0.007	<0.1 ng/mL
	5.0 ng/mL	0.522		100 ng/mL	0.007	<0.1 ng/mL		100 ng/mL	0.007	<0.1 ng/mL
	10 ng/mL	1.001		500 ng/mL	0.004	<0.1 ng/mL		500 ng/mL	0.004	<0.1 ng/mL
	20 ng/mL	1.790		1 μg/mL	0.004	<0.1 ng/mL		1 μg/mL	0.003	<0.1 ng/mL
				5 μg/mL	0.005	<0.1 ng/mL		5 μg/mL	0.004	<0.1 ng/mL

(4) Spike and Recovery

Sample	Average % recovery	Range
EDTA-plasma (n=5)	97.3%	90.0% ~ 108.6%

11. References

- Alles, VV. *et al.* Inducible expression of PTX3, a new member of the pentraxin family, in human mononuclear phagocytes. *Blood*. 1994;84(10):3483-93.
- Introna, M. *et al.* Cloning of mouse ptx3, a new member of the pentraxin gene family expressed at extrahepatic sites. *Blood*. 1996;87(5):1862-72.
- Bottazzi, B. *et al.* Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem*. 1997;272(52):32817-23.
- Wisniewski, HG. and Vilcek, J. Cytokine-induced gene expression at the crossroads of innate immunity, inflammation and fertility: TSG-6 and PTX3/TSG-14. *Cytokine Growth Factor Rev*. 2004;15(2-3):129-46.
- Garlanda, C. *et al.* Pentraxins at the crossroads between innate immunity, inflammation, matrix deposition, and female fertility. *Annu Rev Immunol*. 2005;23:337-66.
- Bottazzi, B. *et al.* The long pentraxin PTX3 as a link among innate immunity, inflammation, and female fertility. *J Leukoc Biol*. 2006;79(5):909-12.
- Polentarutti, N *et al.* Inducible expression of the long pentraxin PTX3 in the central nervous system. *J. Neuroimmunol*. 2000;106(1-2):87-94.
- Salustri, A. *et al.* PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in vivo fertilization. *Development*. 2004;131(7):1577-86.
- Camozzi, M. *et al.* Pentraxin 3 inhibits fibroblast growth factor 2-dependent activation of smooth muscle cells in vitro and neointima formation in vivo. *Arterioscler Thromb Vasc Biol*. 2005;25(9):1837-42.

10. Jeannin, P. *et al.* Complexity and complementarity of outer membrane protein A recognition by cellular and humoral innate immunity receptors. *Immunity*. 2005;22(5):551-60.
11. Jaillon, S. *et al.* The humoral pattern recognition receptor PTX3 is stored in neutrophil granules and localizes in extracellular traps. *J Exp Med*. 2007;204(4):793-804.
12. Breviario, F. *et al.* Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem*. 1992;267(31):22190-7.
13. Vouret-Craviari, V. *et al.* Expression of a long pentraxin, PTX3, by monocytes exposed to the mycobacterial cell wall component lipoarabinomannan. *Infect Immun*. 1997;65(4):1345-50.
14. Rovere, P. *et al.* The long pentraxin PTX3 binds to apoptotic cells and regulates their clearance by antigen-presenting dendritic cells. *Blood*. 2000;96(13):4300-6.
15. Garlanda, C. *et al.* Non-redundant role of the long pentraxin PTX3 in anti-fungal innate immune response. *Nature*. 2002;420(6912):182-6.
16. Nauta, AJ. *et al.* Biochemical and functional characterization of the interaction between pentraxin 3 and C1q. *Eur J Immunol*. 2003;33(2):465-73.
17. Diniz, SN. *et al.* PTX3 function as an opsonin for the dectin-1-dependent internalization of zymosan by macrophages. *J Leukoc Biol*. 2004;75(4):649-56.
18. Han, B. *et al.* TNF α -induced long pentraxin PTX3 expression in human lung epithelial cells via JNK. *J Immunol*. 2005;175(12):8303-11.
19. Baruah, P. *et al.* The pattern recognition receptor PTX3 is recruited at the synapse between dying and dendritic cells, and edits the cross-presentation of self, viral, and tumor antigens. *Blood*. 2006;107(1):151-8.
20. Doni, A. *et al.* Regulation of PTX3, a key component of humoral innate immunity in human dendritic cells: stimulation by IL-10 and inhibition by IFN- γ . *J Leukoc Biol*. 2006;79(4):797-802.
21. Imamura, M. *et al.* Lipopolysaccharide induced expression of pentraxin 3 in human neutrophils and monocyte-derived macrophages. *Cell Immunol*. 2007;248(2):86-94.
22. Kato, S. *et al.* Increased expression of long pentraxin PTX3 in inflammatory bowel diseases. *Dig Dis Sci*. 2008;53(7):1910-6.
23. Savchenko, A. *et al.* Expression of pentraxin 3 (PTX3) in human atherosclerotic lesions. *J Pathol*. 2008;215(1):48-55.
24. Fazzini, F. *et al.* PTX3 in small-vessel vasculitides: an independent indicator of disease activity produced at sites of inflammation. *Arthritis Rheum*. 2001;44(12):2841-50.
25. Latini R. *et al.* Prognostic significance of the long pentraxin PTX3 in acute myocardial infarction. *Circulation*. 2004;110(16):2349-54.
26. Inoue, K *et al.* Establishment of a high sensitivity plasma assay for human pentraxin3 as a marker for unstable angina pectoris. *Arterioscler Thromb Vasc Biol*. 2007;27(1):161-7.
27. Tong, M. *et al.* Plasma pentraxin 3 in patients with chronic kidney disease: associations with renal function, protein-energy wasting, cardiovascular disease, and mortality. *Clin J Am Soc Nephrol*. 2007;2(5):889-97.
28. Kotooka, N. *et al.* Pentraxin3 is a novel marker for stent-induced inflammation and neointimal thickening. *Atherosclerosis*. 2008;197(1):368-74.
29. Kotooka, N. *et al.* Prognostic value of pentraxin 3 in patients with chronic heart failure. *Int J Cardiol*. 2008;130(1):19-22.
30. Suliman, ME. *et al.* Novel links between the long pentraxin 3, endothelial dysfunction, and albuminuria in early and advanced chronic kidney disease. *Clin J Am Soc Nephrol*. 2008;3(4):976-85.
31. Yamasaki K, *et al.* Determination of physiological plasma pentraxin 3 (PTX3) levels in healthy populations. *Clin Chem Lab Med*. 2009;47(4):471-7.

12. Summary of Assay Procedure

