Human PTX3/Pentraxin 3
ELISA

Catalog # LF-EK50795 (1 kit)

Catalog # LF-EK50796 (4 kits bundle)

Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative Detection of human PTX3/Pentraxin 3

For research use only
Not for diagnostic or therapeutic purposes

Contents

1. Introduction ····· 3
2. Principles of Method ····· 3
3. Intended Use ····· 4
4. Storage and Stability ····· 4
5. Chemical Hazard ····· 4
6. Kit Contents ····· 5
7. Materials Required But Not Provided ····· 6
8. Reagent preparation ····· 6
   1) Sample Preparation and Storage ····· 6
   2) Sample Dilution Guideline ····· 7
   3) Reagent Preparation and Storage ····· 7
9. Assay Procedure ····· 8
10. Characteristics ····· 10
   1) Typical result ····· 10
   2) Sensitivity ····· 10
   3) Detection range ····· 10
   4) Specificity ····· 11
   5) Specificity ····· 11
   6) Precision ····· 11
11. Troubleshooting ····· 12
12. Reference ····· 13

1. Introduction

PTX3(Pentraxin 3) is a member of the pentraxin superfamily. This super family characterized by cyclic multimeric structure. The PTX3 gene is mapped to 3q25.32. The predicted 381-amino acid PTX3 protein has homology to the pentraxin protein family. Significant levels of PTX3 were detected in plasma of neutropenic patients with systemic A. PTX3 is effective in preventing CMV infection and reactivation, as well as subsequent Aspergillus infection. PTX3 activates the classical pathway of complementactivation and facilitates pathogen recognition by macrophages and DCs.

2. Principles of Method

Abfrontier's human PTX3 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. A monoclonal antibody from mouse specific for PTX3 has been precoated onto 96-well plates. Standards(NSO, E18-S381) and test samples are added to the wells, a biotinylated detection polyclonal antibody from goat specific for PTX3 is added subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the human PTX3 amount of sample captured in plate.

3. Intended Use

The AbFrontier human PTX3 ELISA kit is to be used for the in vitro quantitative determination of human PTX3 in cell culture supernates, serum, plasma(EDTA) and saliva. The assay will recognize both native and recombinant human PTX3.

This kit has been configured for research use only and is not to be used in diagnostic procedures.

4. Storage and Stability

All kit components of this kit are stable at 2 to 8℃. Any unused reconstituted standard should be discarded or frozen at -20℃. Standard can be frozen and thawed one time only without loss of immunoreactivity.

5. Chemical Hazard

- Stop solution: This reagent is an irritant to eyes, skin and mucous membranes. Avoid contact with eyes, skin and clothing. Wear suitable protective clothing, gloves and eye protection. In the event of contact with eyes or skin, wash immediately with plenty of water.
- All reagents containing Sodium Azide also contain Thimerosal as a preservative. Thimerosal contains Hg thus should be handled with great care.

6. Kit Contents

Contents	Number	Volume
96 Well Plate	1 (in aluminum foil bag with desiccant)	
Standard Protein	2	20 ng/tube
Secondary Antibody	1	130 µl (dilution 1:100)
Avidin-Biotin-Peroxidase Complex (ABC)	1	130 µl (dilution 1:100)
Sample diluent Buffer	1	30 ml
Antibody diluent buffer	1	12 ml
ABC diluent buffer	1	12 ml
TMB color developing agent	1	10 ml
TMB stop solution	1	10 ml

① 96 Well Plate
: Human PTX3 microtiter plate, one plate of 96 wells.
A plate using break-apart strips coated with a monoclonal antibody specific to Human PTX3.

② Standard Protein
: Recombinant Human PTX3.

③ Secondary Antibody
: Biotin labeled anti Human PTX3 antibody.

④ AV-HRP
: Avidin-Biotin-Peroxidase Complex (ABC)

⑤ Substrate (Stabilized chromogen)
: Tetramethylbenzidine (TMB) solution

⑥ Stop Solution
: 1 N solution of sulfuric acid (H_2SO_4)

Notice for Application of Kit

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. The TMB Color Developing agent is colorless and transparent before using, contact us freely if it is not the case.
3. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.

4. Duplicate well assay is recommended for both standard and sample testing.
5. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
6. Don't reuse tips and tubes to avoid cross contamination.
7. To avoid to use the reagents from different batches together.
8. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37℃ for 30 min before using.

7. Materials Required But Not Provided

① Microtiter plate reader in standard size.
② Automated plate washer.
③ Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount.
④ Clean tubes and Eppendorf tubes
⑤ Washing buffer (neutral PBS or TBS).

- Preparation of 0.01M **TBS:** Add 1.2 g Tris, 8.5 g NaCl; 450 ul of purified acetic acid or 700 ul of concentrated hydrochloric acid to 1000 ml H_2O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1 L.
- Preparation of 0.01 M **PBS:** Add 8.5 g sodium chloride, 1.4 g Na_2HPO_4 and 0.2 g NaH_2PO_4 to 1000 ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

8. Reagent Preparation

1) Sample Preparation and Storage

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

- **Cell culture supernates**: Remove particulates by centrifugation, analyze immediately or aliquot and store at -20°C
- **Serum**: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

- **Plasma**: Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 min at 1500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.
- **Saliva:** Collect saliva using a collection device without any protein binding or filtering capabilities such as a Salivette or aliquot and store samples at -20°C.

2) Sample Dilution Guideline

The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice.

The sample must be well mixed with the diluents buffer.

- **High target protein concentration (200-2000 ng/ml).** The working dilution is 1:100. i.e. Add 1µl sample into 99 µl sample diluent buffer.
- **Medium target protein concentration (20-200 ng/ml).** The working dilution is 1:10. i.e. Add 10µl sample into 90 µl sample diluent buffer.
- **Low target protein concentration (312-20,000 pg/ml).** The working dilution is 1:2. i.e. Add 50µl sample to 50 µl sample diluent buffer.
- **Very Low target protein concentration (≤312 pg/ml).** No dilution necessary, or the working dilution is 1:2.

3) Reagent Preparation and Storage

A. Reconstitution of the human PTX3 standard：PTX3 standard solution should be prepared no more than 2 hours prior to the experiment. Two tubes of PTX3 standard (20ng per tube) are included in each kit. Use one tube for each experiment.

a. 20,000pg/ml of human PTX3 standard solution: Add 1ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

b. 10,000pg/ml→312pg/ml of human PTX3 standard solutions: Label 6 Eppendorf tubes with 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312pg/ml respectively. Aliquot 0.3ml of the sample diluent buffer into each tube. Add 0.3ml of the above 20,000pg/ml PTX3 standard solution into 1st tube and mix. Transfer 0.3ml from 1st tube to 2nd tube and mix. Transfer 0.3ml from 2nd tube to 3rd tube and mix, and so on.

Note: The standard solutions are best used within 2 hours. The 20ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

B. Preparation of biotinylated anti-human PTX3 antibody working solution: The solution should be prepared no more than 2 hours prior to the experiment.

- **a.** The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- **b.** Biotinylated anti-human PTX3 antibody should be diluted in 1:100 with the antibody diluent buffer and mixed thoroughly. (i.e. Add 1μl Biotinylated anti-human PTX3 antibody to 99μl antibody diluent buffer.)

C. Preparation of Avidin-Biotin-Peroxidase Complex (ABC) working solution: The solution should be prepared no more than 1 hour prior to the experiment.

- **a.** The total volume should be: 0.1ml/well x (the number of wells). (Allowing 0.1-0.2 ml more than total volume)
- **b.** Avidin- Biotin-Peroxidase Complex (ABC) should be diluted in 1:100 with the ABC dilution buffer and mixed thoroughly. (i.e. Add 1μl ABC to 99μl ABC diluent buffer.)

9. Assay Procedure

The ABC working solution and TMB color developing agent must be kept warm at 37°C for 30 min before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard PTX3 detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of PTX3 amount in samples.

1. Aliquot 0.1ml per well of the 20,000pg/ml, 10,000pg/ml, 5000pg/ml, 2500pg/ml, 1250pg/ml, 625pg/ml, 312pg/ml human PTX3 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add 0.1ml of each properly diluted sample of human cell culture supernates, serum, plasma(EDTA) or saliva to each empty well. **See "Sample Dilution Guideline" above for details.** It is recommended that each human PTX3 standard solution and each sample be measured in duplicate.

2. Seal the plate with the cover and incubate at 37°C for 90 min.

3. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

4. Add 0.1ml of biotinylated anti-human PTX3 antibody working solution into each well and incubate the plate at 37°C for 60 min.

5. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (**Plate Washing Method:** Discard the solution in the

plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 minutes. Repeat this process two additional times for a total of THREE washes. Note: For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)

6. Add 0.1 ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min.

7. Wash plate 5 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 5 for plate washing method).

8. Add 90µl of prepared TMB color developing agent into each well and incubate plate at 37°C in dark for 15-20 min (**Note:** For reference only, the optimal incubation time should be determined by end user. And the shades of blue can be seen in the wells with the four most concentrated human PTX3 standard solutions; the other wells show no obvious color).

9. Add 0.1 ml of prepared TMB stop solution into each well. The color changes into yellow immediately.

10. Read the O.D. absorbance at 450nm in a microplate reader within 30 min after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The human PTX3 concentration of the samples can be interpolated from the standard curve.

Note: if the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Summary

1. Add samples and standards and incubate the plate at 37°C for 90 min. Do not wash.
2. Add biotinylated antibodies and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS.
3. Add ABC working solution and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS.
4. Add TMB color developing agent and incubate the plate at 37°C in dark for 15-20 min.
5. Add TMB stop solution and read.

10. Characteristics

1) Typical result

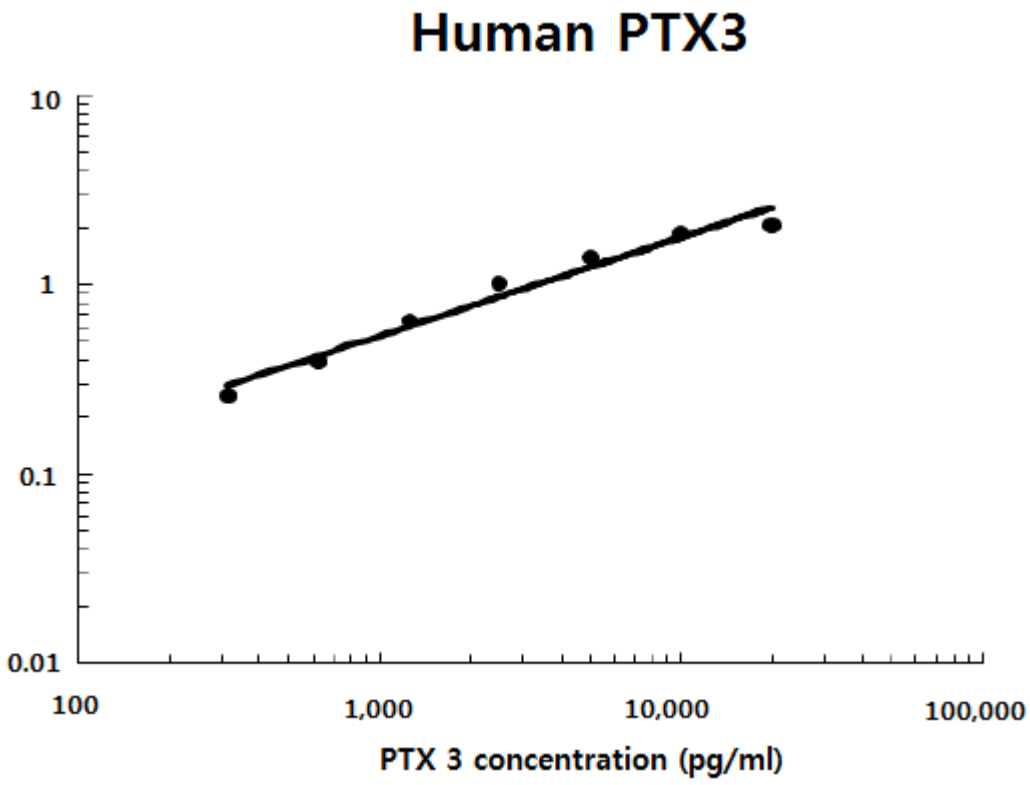
Typical Data Obtained from Human PTX3

(TMB reaction incubate at 37℃ for 15 min)

Standard Human PTX3 (pg/ml)	Optical Density (at 450nm)
0	0.072
312	0.260
625	0.394
1,250	0.647
2,500	1.029
5,000	1.399
10,000	1.883
20,000	2.063

Typical Human PTX3 ELISA Kit Standard Curve

This standard curve was generated at AbFrontier for demonstration purpose only. A standard curve must be run with each assay.



2) Sensitivity: < 10 pg/ml

3) Detection range: 312pg/ml-20,000pg/ml

4) Specificity: No detectable cross-reactivity with other relevant proteins

5) Specificity: Natural and recombinant human PTX3

6) Precision

Intra-Assay Precision (Precision within an assay): Three samples of known concentration were tested 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.

	Intra-Assay Precision			**Inter-Assay Precision**		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
N	16	16	16	24	24	24
Mean(ng/ml)	3.5	7.8	15.7	3.86	8.1	15.3
Standard deviation	0.22	0.45	1.0	0.28	0.48	1.16
CV(%)	6.3	5.8	6.4	7.3	5.9	7.6

11. Troubleshooting

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much AV-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20~30 ℃ before performing assay
Too much signal – whole plate turned uniformly blue	• Insufficient washing – unbound AV-HRP remaining	• Increase number of washes carefully
	• Too much AV-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual AV-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

12. Reference

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