HIGH SENSITIVITY HUMAN PENTRAXIN 3 (PTX3) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN PTX3 CONCENTRATIONS IN SERUM AND EDTA PLASMA

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITENS OF EACH KIT BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:
THIS KIT IS FOR ONE TIME USE ONLY.

ELISA NAME	**HIGH SENSITIVITY HUMAN PTX3 ELISA KIT**
Catalog No.	SK00101-06
Lot No.	20114482
Formulation	96 T
Standard range	31.25-2000 pg/mL
Sensitivity	7 pg/mL
Sample Volume	100 µL
Sample Type	Serum, EDTA Plasma
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Specificity	Human PTX3
Calibration	Human PTX3 recombinant (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	4 - 10%
Storage	2 – 8° C for 4 months. More information check page 2-3
This kit contains sufficient materials to run approximately 35-40 samples duplicated provided that assay is run according to protocol.	

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DESCRIPTION

This High Sensitivity Human PTX3 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human PTX3 from serum and plasma in a sandwich ELISA format.

This immunoassay contains recombinant human PTX3 (HEK293 derived) and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural PTX3 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human PTX3. The capture antibody can bind to the human Neprilysin in the standard and samples. After washing the plate of any unbound substances, a biotinylated monoclonal antibody against human PTX3 is added to the wells. After another washing of the plate, Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of human PTX3 bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
PTX3 Microplate - 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against Human PTX3.	**101-06-01**	**1 plate**
PTX3 Standard – 4800 pg/vial of recombinant human PTX3 in a buffered protein base with preservative; lyophilized.	**101-06-02**	**1 vial**
Detection Antibody Concentrate – 1.2 mL/vial, 10-fold concentrate of biotinylated monoclonal antibody against human PTX3 with preservative; lyophilized.	**101-06-03**	**1 vial**
Positive Control - one vial of recombinant human PTX3; lyophilized.	**101-06-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial, 100-fold concentrated solution of Streptavidin conjugate to HRP.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered protein based solution with preservative.	**DB06**	**1 bottle**
Antibody Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB10**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered protein based solution with preservative.	**DB08B**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution -11 mL of TMB substrate solution.	**TMB03**	**1 bottle**
Stop Solution – 11 mL of 0.25M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8° C for up to 4 months. For long-term storage up to 10 months, place unopened Standard, Positive Control, and Detection Antibody Concentrate, Dilution Buffer and Antibody & HRP Diluent Solution should be stored at -20° C. Streptavidin-HRP Conjugate and TMB Substrate Solution should be stored only at 2 – 8° C. Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (**400-450 rpm**).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes 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.

SAMPLE PREPARATION

Serum or EDTA plasma samples may need to be diluted by 2 ~ 4. **Optimal dilutions; however, should be determined by each laboratory for each application.**
Use polypropylene test tubes.

REAGENT PREPARATION

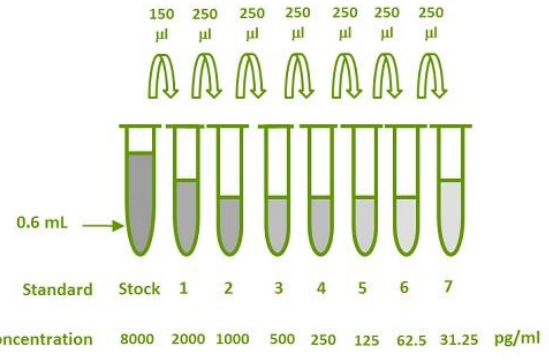
Bring all reagents to room temperature before use.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of 1x Wash Buffer.

Dilution Buffer (DB06) - **If Dilution Buffer is highly viscous, warm in 27 - 30° C water bath until liquid flows more freely.**

Human PTX3 Standard - Reconstitute the Neprilysin fc standard with 0.6 mL of **Dilution Buffer (DB06)**. This reconstitution produces a stock solution of 8000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 450 µL of Dilution Buffer (DB06) into tubes #1. Pipette 250 µL of Dilution Buffer (DB06) to #2 to #7. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The **2000 pg/mL** standard serves as the high standard. The Dilution Buffer (DB06) serves as the zero standard (0 pg/mL). *Store the stock solution of standard (8000pg/mL) at -20 ~ -70 °C for a few days.*

TUBE	STANDARD	DILUTION BUFFER (DB06)	CONCENTRATION
stock	Powder	0.6 ml	8000 pg/ml
# 1	150 µl of stock	450 µl	2000 pg/ml
# 2	250 µl of 1	250 µl	1000 pg/ml
# 3	250 µl of 2	250 µl	500 pg/ml
# 4	250 µl of 3	250 µl	250 pg/ml
# 5	250 µl of 4	250 µl	125 pg/ml
# 6	250 µl of 5	250 µl	62.5 pg/ml
# 7	250 µl of 6	250 µl	31.25 pg/ml



Positive Control - Reconstitute the Positive Control with 1.0 mL of **Dilution Buffer (DB06)**. Discard the positive control solution after use. It is for one time use only.

Detection Antibody Concentrate - Reconstitute the Detection Antibody Concentrate with 1.2 mL of **Antibody Diluent Solution (DB10)** to produce a 10-fold concentrated stock solution. For 96 wells test, freshly pipette 9.45 mL of **Antibody Diluent Solution (DB10)** into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

If run partial 8-well strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution of 10-fold concentrated detection antibody at -20 °C for a few days.

Streptavidin-HRP Conjugate – For 96 wells test, freshly pipette 11.88 mL of **HRP Diluent Solution (DB08B)** into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Protect from light.** The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within 20-30 min.

If run partial 8-well strip test, freshly prepare 900 µL per strip of working solution. Store the stock solution of 100-fold concentrated Streptavidin HRP at 2 - 8 °C for 10 months.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Add 100 µL of **Dilution Buffer (DB06)** to Blank wells.
3. Add 100 µL of **Standard dilutions** in reverse order of serial dilution, **samples**, or **positive control** per well. Cover with plate sealer. Incubate for **2 hours** on microplate shaker (400-450 rpm) at room temperature.
4. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with **1x Wash Buffer** (300 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.
5. Add 100 µL of **Detection Antibody working solution** to each well. Cover with plate sealer. Incubate for 2 hours on microplate shaker at room temperature.
6. Repeat the aspiration/wash as in step 4.
7. Add 100 µL of **Streptavidin-HRP Conjugate working solution** to each well. Incubate for 60 minutes on microplate shaker at room temperature. **Protect from light.**
8. Repeat the aspiration/wash as in step 4.
9. Add 100 µL of **Substrate Solution** to each well. Incubate for 12-17 minutes on microplate shaker at room temperature. **Protect from light.**
10. Add 100 µL of **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 if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well using a microplate reader set to 450 nm within 3 min.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control and sample, and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log or 4-Parameter curve fit.

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

SPECIFICITY

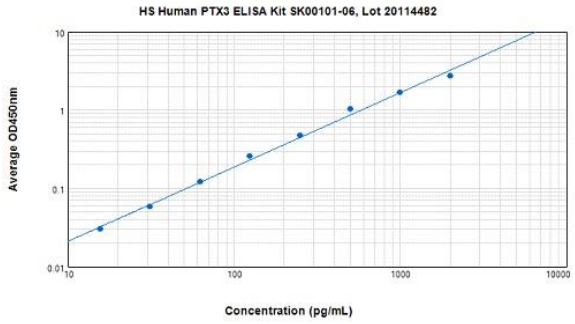
PROTEINS	CROSS-REACTIVITY (%)
Human Pentraxin 3 (HEK293)	100
Mouse Pentraxin 3 (HEK293)	0
Rat Pentraxin 2	0
Mouse Pentraxin 2	0

TYPICAL DATA

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

STANDARD (PG/ML)	AVERAGE OD450NM (CORRECTED)
Blank	0 (0.078)
15.6 (optional)	0.030
31.25	0.059
62.5	0.120
125	0.257
250	0.469
500	1.029
1000	1.679
2000	2.726

- Lot: 20114482
- Positive control: 200 - 800 pg/mL (log-log fit)



SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard dilutions, samples, or positive control to the well. Incubate 2 hours on the plate shaker (400-450 rpm) at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Detection Antibody working solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to each well. Incubate 60 minutes on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 12-17 min on the plate shaker at RT. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read at 450nm within 3 min.