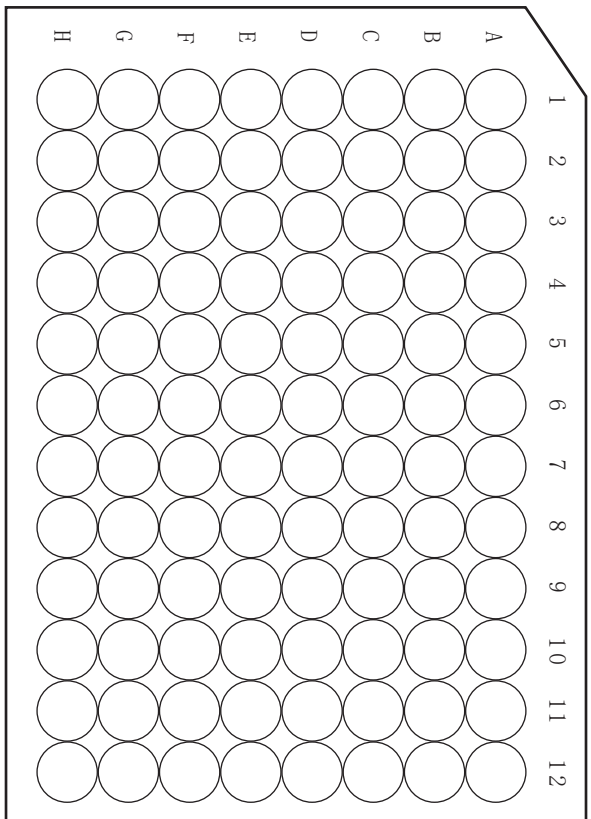




DESCRIPTION

# Human Pentraxin 3 Immunoassay

Catalog Number: SEKH-0293

For the quantitative determination of Human Pentraxin 3 concentrations in cell culture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

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**LINEARITY:**To assess the linearity of the assay, three samples were spiked with high concentrations of human Pentraxin 3 in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

| Dilution ratio | Recovery(%) | Citrate plasma | Cell culture supernatants |
|---|---|---|---|
| 1:2 | Average% of Expected | 98 | 108 |
| | Range(%) | 97—119 | 101—114 |
| 1:4 | Average% of Expected | 100 | 109 |
| | Range(%) | 98—115 | 101—115 |
| 1:8 | Average% of Expected | 98 | 105 |
| | Range(%) | 89-109 | 99-115 |
| 1:16 | Average% of Expected | 101 | 104 |
| | Range(%) | 94-110 | 95-117 |

## Performance Characteristics

**SENSITIVITY:** The minimum detectable dose was 19.5pg/mL.
**SPECIFICITY:** This assay recognizes both natural and recombinant Human Pentraxin 3. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

| Recombinant Human | Recombinant mouse | Recombinant rat |
|---|---|---|
| Pentraxin 2 | Pentraxin 2 | Pentraxin 2 |
| | | |
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**REPEATABILITY:** The coefficient of variation of both intra-assay and inter-assay were less than 10%.
**RECOVERY:** The recovery of Human Pentraxin 3 spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of Human Pentraxin 3 in two matrices

| Sample Type | Average % of Expected Range(%) | Range(%) |
|---|---|---|
| Citrate plasma | 89 | 81—95 |
| Cell culture supernatants | 104 | 96—114 |



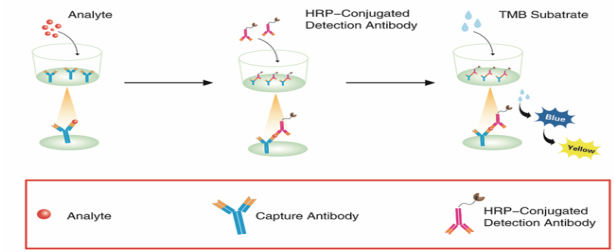


## BACKGROUND

Pentraxin 3 (PTX3), also known as TNF-stimulated gene 14 (TSG-14), is a secreted glycoprotein belonging to the Pentraxin family of proteins. It is the prototypical long Pentraxin, exhibiting a C-terminal Pentraxin domain characteristic of the family, and a unique N-terminal domain. It is produced by several cell types including endothelial and mononuclear cells. PTX3 acts as a pattern recognition receptor with non-redundant roles in the innate immune response to several microbial agents including the fungal pathogen Aspergillus fumigatus. Overexpression leads to enhanced pro-inflammatory responses. PTX3 is induced in response to LPS and inflammatory cytokines including TNF-alpha and IL-1 beta.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for Pentraxin 3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells; any Pentraxin 3 present is captured by the coated antibody after incubation. After washing away any unbound substances, a biotin-conjugate antibody specific for Pentraxin 3 is added to detect the captured Pentraxin 3 protein in the sample. Following a wash to remove any unbound combination, horseradish peroxidase (HRP)-conjugated Streptavidin is added to the wells. After extensive washing, a tetramethyl-benzidine (TMB) reagent is added to the wells for signal development. Solution containing sulfuric acid is used to stop color development. The color intensity, proportional to the quantity of bound protein, is then measurable at 450nm.




## TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

## PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

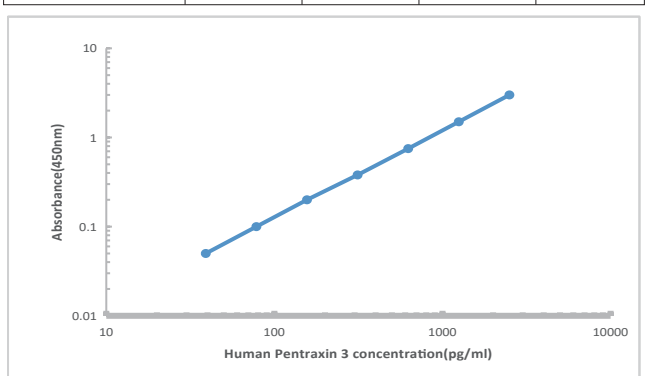




determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
5.This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the Human Pentraxin 3 ELISA

| Standared(pg/ml) | OD. | OD. | Average | Corrected |
|---|---|---|---|---|
| 0 | 0.021 | 0.015 | 0.018 | - |
| 39.06 | 0.075 | 0.196 | 0.135 | 0.117 |
| 78.1 | 0.118 | 0.285 | 0.201 | 0.183 |
| 156 | 0.207 | 0.424 | 0.315 | 0.297 |
| 312.5 | 0.392 | 0.673 | 0.533 | 0.515 |
| 625 | 0.685 | 1.093 | 0.889 | 0.871 |
| 1250 | 1.291 | 1.780 | 1.535 | 1.517 |
| 2500 | 2.439 | 2.900 | 2.669 | 2.651 |




Representative standard curve for Human Pentraxin 3 ELISA.

## ASSAY PROCEDURE

Prepare all reagents and standards as directed. Wash the plate 3 times before assay.

⇩

Add 100µl standard or samples to each well, shaking with Micro-oscillator (100r/min) to incubate 120 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 4 times

Add 100µl working solution of HRP-Conjugate anti- human Pentraxin 3 antibody to each well, shaking with Micro-oscillator (100r/min) to incubate 60 minutes at room temperature(25±2℃).

⇩ Aspirate and wash 5 times

Add 100µl Substrate solution to each well, incubate 5-30 minutes (depending on signal) at room temperature(25±2℃).Protect from light.

⇩

Add 50µl Stop solution to each well. Read at 450nm within 5 minutes.

## CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample. All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct 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.
4. The data may be linearized by plotting the log of the Human Pentraxin 3 concentrations versus the log of the O.D. and the best fit line can be





## KIT COMPONENTS& STORAGE CONDITIONS

| PART | SIZE | STORAGE OF OPENED/ RECONSTITUTED MATERIAL |
|---|---|---|
| **Microwell Plate**-antibody coated 96-well Microplate (8 wells ×12 strips) | 1 plate | 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** |
| **Standard** - lyophilized,2500pg/ml upon reconstitution | 2 vials | Store at 2-8°C** for six months |
| **HRP Congugated Antibody（100×）** - 120 ul/vial | 1 vial | Store at 2-8°C** for six months |
| **Standard /sample Diluent** - 16ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **HRP Congugated Diluent** - 16ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Wash Buffer Concentrate** (20x) - 30 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Substrate Solution** - 12 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Stop Solution** - 12 ml/vial | 1 bottle | Store at 2-8°C** for six months |
| **Plate Cover Seals** | 4 pieces | |
| | | |

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

## OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

## SPECIMEN COLLECTION & STORAGE

**Cell Culture Supernates** - Centrifuge cell culture media at 1000×g to remove debris. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
**Serum** - Use a serum separator tube (SST) and allow samples to clot for 2 hours at room temperature or overnight at 2-8℃. Centrifuge approximately for 15 minutes at 1000×g. 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×g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.
Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

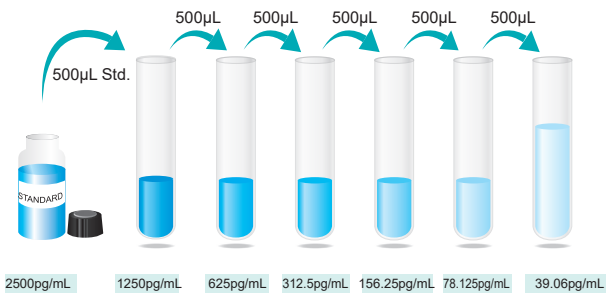
## REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentrate with 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard\Specimen** - Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution





of 2500pg/mL.Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 500uL of Standard/Sample Diluent into 1250pg/ml tube and the remaining tubes. Use the stock solution of 2500pg/mL to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2500pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).




Preparation of **Pentraxin 3** standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of HRP-Congugated Antibody(100×):Make a 1:100 dilution in Reagent Diluent. If the entire 96-well plate is used, add 100uL of HRP Conjugate to 10mL of HRP-Congugated Diluent to make working dilution of HRP Conjugate and mix thoroughly prior to the assay. The rest of undiluted HRP Conjugate can be stored at 4°C for up to 6 months. DO NOT FREEZE**
***The working solution should be used within one day after dilution.**