Pancreatic Polypeptide ELISA

For the quantitative determination of pancreatic polypeptide (PP) in human serum, plasma, supernatant, and tissue samples.

For Research Use Only. Not for Use in Diagnostic Procedures.

**Ship and Store the Human PP Antibody-Coated Microplate, Human PP Standard, Biotinylated PP, and Streptavidin-HRP Conjugate at -20°C.*

Catalog Number: 07-PPPHU-E01

Size: 96 determinations

Version: 1.0 **ALPCO**: 1.0

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INTENDED USE

The Pancreatic Polypeptide ELISA is an enzyme-linked immunoassay for the quantitative determination of pancreatic polypeptide (PP) in human serum, plasma, supernatant, and tissue samples. For Research Use Only. Not for use in diagnostic procedures.

PRINCIPLE OF THE ASSAY

The method employs competitive enzyme-linked immunosorbent assay (ELISA) technique to measure the level of Pancreatic Polypeptide, PP, in samples. Standards or samples compete with the biotinylated PP to form a complex with the anti-PP antibody-coated microtiter well. Wells are washed to remove the excess conjugate and Streptavidin-HRP Conjugate is added to the microplate and incubated. After incubation and a washing step, TMB Substrate is added. Blue color develops upon incubation, and the reaction is stopped with a Stop Solution to form a yellow color. The concentration of the Pancreatic Polypeptide (PP) in the samples is inversely proportional to the yellow color (absorbance) in the wells.

MATERIALS SUPPLIED

07-PPPHU-E01			
Component	**Composition**	**Quantity**	**Preparation**
*Human PP Antibody-Coated Microtiter Plate	96 wells	1	Ready-to-Use
*Human PP Standard	30,000 pg/mL	2 vials	Lyophilized
*Biotinylated PP	120 uL	1 vial	Concentrated
*Streptavidin-HRP Conjugate	120 uL	1 vial	Concentrated
Standard Diluent	20 mL	1 bottle	Ready-to-Use
Biotin Antigen Dilution Buffer	12 mL	1 bottle	Ready-to-Use
HRP Conjugate Dilution Buffer	12 mL	1 bottle	Ready-to-Use
20X Wash Buffer	25 mL	1 bottle	Concentrated
TMB Substrate	12 mL	1 bottle	Ready-to-Use
Stop Solution	12 mL	1 bottle	Ready-to-Use

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ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter Plate Reader able to measure absorbance at 450 nm.
- Adjustable pipettes and multichannel pipettor to measure volumes ranging from 25 ul to 1000 ul
- Deionized (DI) water
- Wash bottle or automated microplate washer
- Tubes and Eppendorf tubes
- Precision single and multi-channel pipette and disposable tips
- 37°C incubator
- Timer

PRECAUTIONS

- This kit is For Research Use Only.
- Follow the working instructions carefully.

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- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shifts during pipetting of reagents.
- All reagents should be kept in the original shipping containers.
- Some of the reagents contain a small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
    - Do not smoke, eat, or drink while handling kit material
    - Always use protective gloves
    - Never pipette material by mouth
    - Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case Good Laboratory Practice should be applied with all general and individual regulations to the use of this kit.
- Reagents that contain preservatives may be harmful if ingested, inhaled, or absorbed through the skin.

REAGENT HANDLING and STORAGE CONDITIONS

1. Store all reagents as indicated on the component label: Store the Human PP Antibody-Coated Microplate, Human PP Standard, Biotinylated PP, and Streptavidin-HRP Conjugate at **-20°C**. Store the rest of the reagents at **2 - 8°C** as per the label.
2. TMB Substrate is light-sensitive; protect from direct sunlight and UV sources.
3. Use all reagents and wash solutions within 12 months of manufacturing date.
4. Before use, bring all components to room temperature (18-25ºC). Upon assay completion, ensure all kit components are returned to appropriate storage conditions.
5. The substrate is light-sensitive and should be protected from direct sunlight or UV sources.

SAMPLE PREPARATION AND STORAGE

Samples should be clear and non-hemolyzed. Samples should be run at several dilutions to ensure accurate quantitation.

Process samples as quickly as possible after collection. Samples should be tested as soon as possible after processing. Alternately, samples can be kept at -20°C. Avoid repeated freeze-thaw cycles.

1. Serum

Coagulate at room temperature for 10-20 minutes. Centrifuge for 20 min. at 2,000-3,000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.

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2. Plasma

Use EDTA or citrate as an anticoagulant, mix for 10-20 minutes. Centrifuge for 15 min. at 2,000-3,000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

3. Cell Culture Supernatant

Collect sample in a sterile container. Centrifuge for 20 min. at 2,000-3,000 rpm. Remove the supernatant carefully. When examining the components within the cell, dilute cell suspension with PBS (pH 7.2 - 7.4), if cell concentration is greater than 1 million cells/ml. Lyse the cells via repeated freeze-thaw cycles to release intracellular components. Centrifuge for 20-min at 2,000-3,000 rpm. If precipitation appears, centrifuge again.

4. Tissue Samples

Rinse tissues in PBS (pH 7.4) to remove excess blood thoroughly, and weigh before homogenization. Mince tissues and homogenize them in PBS (pH7.4) with a glass homogenizer on ice. Thaw at 2-8°C or freeze at -20°C. Centrifuge at 2,000-3,000 rpm for approximately 20 minutes and collect the supernatant carefully.

Note: Grossly hemolyzed samples are not suitable for use in this assay.

REAGENT PREPARATION

All reagents should be diluted immediately prior to use.

Label any aliquots with the kit lot number and expiration date and store at indicated temperature and conditions.

Bring all reagents to room temperature before use.

1. **To make 1X working Wash Buffer**:

   Dilute 25 ml of (20X) Wash Buffer in 475 ml of DI water.

2. **To make 1X working Streptavidin:HRP Conjugate**:

   Briefly spin or centrifuge the streptavidin:HRP Conjugate before use. Dilute 100-fold with HRP Conjugate Dilution Buffer

3. **To make 1X working Biotinylated PP Working Solution:**

   Briefly spin or centrifuge the Biotinylated PP before use. Dilute 100-fold with Biotin Antigen Dilution Buffer.

4. **Standards Preparation**:

   Reconstitute original Pancreatic Polypeptide, PP, Standard with 1.0 ml of Standard Diluent. Let sit for 10 mins with gentle agitation before making further dilutions. Prepare the additional Standards by serially diluting the standard stock solution as per the table below.

Standard Concentration	Standard Vial	Preparation Instructions
30,000 pg/mL	Original Standard	Reconstitute with 1.0 mL Standard Diluent
3,000 pg/mL	Standard #6	300 µL Original Standard + 600 µL Standard Diluent

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1,000 pg/mL	Standard #5	300 µL Standard #6 + 600 µL Standard Diluent
333 pg/mL	Standard #4	300 µL Standard #5 + 600 µL Standard Diluent
111 pg/mL	Standard #3	300 µL Standard #4 + 600 µL Standard Diluent
37 pg/mL	Standard #2	300 µL Standard #3 + 600 µL Standard Diluent
0 pg/mL	Standard #1	600 µL Standard Diluent only

QUALITY CONTROL

It is recommended that each laboratory assay appropriate quality control samples with each run to ensure that all reagents and procedures are correct.

PROCEDURAL NOTES

1. To achieve good assay reproducibility and sensitivity, proper washing of the plates to remove excess reagents is essential.
2. High Dose Hook Effect may be observed in samples with very high concentrations of human pancreatic polypeptide. High Dose Hook Effect can occur when an excess of antibody is coupled with very high concentrations of human pancreatic polypeptide in the sample.

   If PP concentration in an undiluted sample is less than the diluted sample, this may be indicative of a hook effect.
3. Avoid assay of samples containing sodium azide (NaN3), as it could destroy the HRP activity resulting in underestimation of the PP concentration.
4. It is recommended that all Standards and Samples be assayed in duplicate or triplicate.
5. Maintain a repetitive timing sequence from well to well for all the steps to ensure that the incubation timings are the same for each well.
6. If the Substrate has a distinct blue color prior to use, it may have been contaminated and use of such substrate can lead to inaccurate results.
7. The plates should be read within 15 minutes after addition of the Stop Solution.
8. Make a plate map to identify the location of standards and samples.

ASSAY PROCEDURE

It is strongly recommended that all Standards and Samples be run in duplicate or triplicate. A standard curve is required for each assay.

1. Add **50 µl prepared Standards and Samples** to respective wells.
2. Pipette **50 µl 1X working Biotinylated PP Solution** to all wells.
3. Cover the plate with a sealer and incubate for 60 minutes at 37°C.
4. Aspirate and wash plate 4 times with 1X working Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe off any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.
5. Pipette **100 µl 1X working Streptavidin HRP Conjugate** into all wells. Mix well.

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6. Cover the plate with a sealer and incubate for 30 minutes at 37°C.
7. Aspirate and wash as per Step (4) above.
8. Pipette **100 µl TMB Substrate** into all the wells.
9. Incubate the plate at **37°C** for **10 minutes**. DO NOT SHAKE. Shaking may result in higher background and poor precision.
10. Pipette **100 µl** of **Stop Solution** into all wells. The wells should turn from blue to yellow in color.
11. Read the absorbance at 450 nm with a calibrated microplate reader within 10-15 minutes after addition of Stop solution.

CALCULATION OF RESULTS

Determine the mean absorbance for each set of duplicate or triplicate Standards and Samples.

For manual calculation, use graph paper to plot the average value (absorbance 450 nm) of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis. Draw the best fit curve through the standard points. To determine the unknown PP concentrations, find the unknown's mean absorbance value on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the PP concentration.

If samples were diluted, multiply by the appropriate dilution factor.

Note: It is recommended to repeat the assay at a higher dilution factor if the sample absorbance value is below the first standard.

This is a competitive assay. Low signal correlates to high PP concentrations.

PERFORMANCE CHARACTERISTICS

Assay Range

37 pg/ml – 3,000 pg/ml

Sensitivity

Limit Of Quantification (LOQ) is defined as the lowest detectable concentration that can be determined with acceptable repeatability.

The LOQ was found to be 15.3 pg/ml.

Specificity

This assay has high sensitivity and excellent specificity for detection of pancreatic polypeptide (PP). No significant cross-reactivity or interference between pancreatic polypeptide (PP) and analogues was observed.

Precision

Intra-Assay: CV<10%
Inter-Assay: CV<12%

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Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of PP and their serial dilutions. The results are expressed as percentage of calculated concentration to the expected concentration.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	96-103%	80-93%	81-97%	88-107%
EDTA Plasma (n=5)	82-96%	95-108%	84-98%	86-95%
Heparin Plasma (n=5)	91-101%	88-104%	85-95%	78-92%

Spike and Recovery

Matrices listed below were spiked with a certain level of pancreatic polypeptide (PP) and the recovery rates were calculated by comparing the measured value to the expected amount of PP in samples.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	90 - 103	95
EDTA plasma (n=5)	80 - 94	87
Heparin Plasma (n=5)	82 - 98	93

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