Human Pancreatic Polypeptide (PP)

96-Well Plate

Cat. # EZHPP40K, EZHPP-40BK

HUMAN PANCREATIC POLYPEPTIDE (PP) ELISA KIT

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I. INTENDED USE

This Human Pancreatic Polypeptide (PP) ELISA kit is used for the non-radioactive quantification of Human PP in human serum, plasma, tissue extract and cell culture samples. One kit is sufficient to measure 39 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

II. PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of human PP molecules from samples to the wells of a microtiter plate coated by a pre-titered amount of anti-human PP polyclonal antibody, 2) wash away of unbound materials from samples, 3) binding of a second biotinylated anti-PP polyclonal antibody to the captured molecules, 4) wash away of unbound materials from samples, 5) conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 6) wash away of free enzyme conjugates, and 7) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590 nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured human PP in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human PP.

III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

A. Human PP ELISA Plate

Coated with anti-Human PP Polyclonal Antibodies
Quantity: 1 strip plate
Preparation: Ready to Use
Note: Unused strips should be resealed in the foil pouch with the dessicant provided.

B. Adhesive Plate Sealer

Quantity: 2 sheets
Preparation: Ready to Use

C. 10X Concentrate HRP Wash Buffer

10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.
Quantity: 2 bottles containing 50 ml each
Preparation: Dilute 1:10 with distilled or deionized water.

III. REAGENTS SUPPLIED (continued)

D. Human PP Standard
Synthetic Human PP, lyophilized.
Quantity: 0.5 mL/vial upon hydration
Preparation: Reconstitute with 0.5 mL distilled or deionized water.

E. Human PP Quality Controls 1 and 2
One vial each, lyophilized, containing synthetic Human PP at two different levels.
Quantity: 0.5 mL/vial upon hydration
Preparation: Reconstitute with 0.5 mL distilled or deionized water.

F. Assay Buffer
0.05 Phosphosaline, containing 0.025M EDTA, 0.08% Sodium Azide, 1.0% BSA
Quantity: 40 mL
Preparation: Ready to Use

G. Human PP Detection Antibody
Pre-titered Biotinylated Goat anti-Human PP Polyclonal Antibody
Quantity: 12 mL
Preparation: Ready to Use

H. Enzyme Solution
Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer
Quantity: 12 mL
Preparation: Ready to Use

I. Substrate (Light sensitive, avoid unnecessary exposure to light)
3, 3', 5, 5'-tetramethylbenzidine in buffer
Quantity: 12 mL
Preparation: Ready to Use.

J. Stop Solution (Caution: Corrosive Solution)
0.3 M HCl
Quantity: 12 mL
Preparation: Ready to Use

K. Matrix Solution
Quantity: 1 mL/vial, lyophilized
Preparation: Reconstitute with 1.0 mL distilled or deionized water.

IV. STORAGE AND STABILITY

- Recommended storage for kit components is 2-8°C.

- All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use but repeated freeze thaws should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

V. REAGENT PRECAUTIONS

A. Sodium Azide

Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

B. Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

Note: See Full Labels of Hazardous components on next page.

Full labels of hazardous components in this kit:

Ingredient, Cat #		Full Label	
Human Pancreatic Polypeptide Detection Antibody	E1040		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Human Pancreatic Polypeptide Quality Control 1 & 2	E6040K		**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Human Pancreatic Polypeptide Standard	E8040K		**Danger.** Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention.
Assay Buffer	EAB-P		**Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Matrix Solution	EMTXPP	No Symbol Required	Harmful to aquatic life with long lasting effects. Avoid release to the environment.
Stop Solution	ET-TMB		**Warning.** May be corrosive to metals.
10X HRP Wash Buffer Concentrate	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 10 µL - 20 µL or 20 µL - 100 µL
2. Multi-Channel Pipettes and Pipette Tips: 5 ~ 50 µL and 50 ~ 300 µL
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Deionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth

VII. SAMPLE COLLECTION AND STORAGE

1. To prepare serum samples, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Let blood clot at room temperature for 30 min.

   Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2°C.

   Transfer and store serum samples in separate tubes. Date and identify each sample.

   Use freshly prepared serum or aliquot and store samples at ≤ –20°C for later use. For long-term storage, keep at -70°C. Avoid freeze/thaw cycles.

2. To prepare plasma samples, whole blood should be collected into centrifuge tubes containing enough K_3EDTA to achieve a final concentration of 1.735 mg/mL and centrifuged immediately after collection. Observe the same precautions in the preparation of serum samples.

3. If heparin is to be used as an anticoagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.

4. Avoid using samples with gross hemolysis or lipemia.

VIII. SAMPLE PREPARATION

1. No dilution or preparation is needed for normal serum or plasma samples. In the event that any sample is above 3000 pg/mL range, dilutions should be performed using the Serum Matrix provided.

2. Tissue extracts or cell culture media samples may require dilution. Dilutions should be performed using the assay buffer provided.

IX. REAGENT PREPARATION

A. Human PP Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human PP Standard with 0.5 mL distilled or deionized water to give a concentration described in the analysis sheet. Invert and mix gently, and let sit for 5 minutes then mix well.

2. Label five tubes 1, 2, 3, 4, and 5. Add 200 µL Assay Buffer to each of the five tubes. Perform 3 time serial dilutions by adding 100 µL of the reconstituted standard to Tube 1, mix well and transfer 100 µL of Tube 1 to Tube 2, mix well and transfer 100 µL of Tube 2 to Tube 3, mix well and transfer 100 µL of Tube 3 to Tube 4, mix well and transfer 100 µL of Tube 4 to Tube 5 and mix well.

   Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of reconstituted standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Concentration pg/mL
0.5 mL	0	X (refer to analysis sheet For exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
1	200 µL	100 µL of reconstituted Standard	X/3
2	200 µL	100 µL of Tube 1	X/9
3	200 µL	100 µL of Tube 2	X/27
4	200 µL	100 µL of Tube 3	X/81
5	200 µL	100 µL of Tube 4	X/243

B. Human PP Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Human PP Quality Control 1 and Quality Control 2 with 0.5 mL distilled or deionized water into the vials. Invert and mix gently, let sit for 5 minutes then mix well.

C. Matrix Solution Preparation

Use care in opening the lyophilized Matrix Solution. Using a pipette, reconstitute the Matrix Solution with 1.0 mL distilled or deionized water. Invert and mix gently, let sit for 5 minutes then mix well.

X. ASSAY PROCEDURE

Pre-warm all reagents to room temperature prior to setting up the assay.

1. Dilute the 10X concentrated Wash Buffer 10 fold by mixing the entire content of each bottle of wash buffer concentrate with 450 ml deionized or distilled water (dilute both buffer bottles with 900 mL deionized or distilled water).

2. Remove the required number of strips from the Microtiter Assay Plate. Assemble the strips in an empty plate holder and fill each well with 300 µL of diluted Wash Buffer. Incubate at room temperature for 5 minutes. Decant Wash Buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. **Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, use a gentle wash program for all washing steps described in this protocol.**

3. Add in duplicate, 50 µL Assay Buffer to the blank wells and sample wells.

4. Add 50 µL Matrix Solution to Blank wells, Standard wells, and QC1 and QC2 wells.

5. Add in duplicate, 50 µL Human PP Standards in the order of ascending concentration to the appropriate wells. Add in duplicate, 50 µL QC1 and 50 µL QC2 to the appropriate wells. Add sequentially, 50 µL of the unknown samples in duplicate to the remaining wells. **For best result all additions should be completed within 30 minutes.**

6. Cover the plate with plate sealer and incubate at room temperature for 1.5 hours on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

7. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

8. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

9. Add 100 µL Detection Antibody to all wells. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400 to 500 rpm.

10. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in the wells.

11. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

X. ASSAY PROCEDURE (continued)

12. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

13. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid.

14. Wash wells 3 times with 1X Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

15. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for 5 to 20 minutes (A longer development time may be needed if using a plate washer). Blue color should be formed in wells of PP standards with intensity proportional to increasing concentrations of PP.

    Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

16. Remove sealer and add 100 µL Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest PP standard should be approximately 2 - 3.2, or not to exceed the capability of the plate reader used.

Assay Procedure for Human PP ELISA Kit (EZHPP40K)

	Step 1	Step 2	Step 3	Step 4	Step 5	Step 6-8	Step 9	Step 10-11	Step 12	Step 13-14	Step 15	Step 15	Step 16	Step 16
Well #	**Dilute each bottle of 10X Wash Buffer with 450 ml Deionized Water.**	**Wash plate with 300 µL Wash Buffer and Incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels**	Assay Buffer	Matrix Solution	Standards/ Controls/ Samples	**Seal, Agitate, Incubate 1.5 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer**	Detection Ab	**Seal, Agitate, Incubate 1 hour at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels. Wash 3X with 300 µL Wash Buffer**	Enzyme Solution	**Seal, Agitate, Incubate 30 minutes at Room Temperature . Wash 3X with 300 µL Wash Buffer**	Substrate	**Seal, Agitate, Incubate 5-20 minutes at Room Temperature.**	Stop Solution	**Read Absorbance at 450 nm and 590 nm.**
A1, A2			50 µL	50 µL	---------		100 µL		100 µL		100 µL		100 µL	
A3, A4			--	50 µL	50 µL of Tube 5									
A5, A6			--	50 µL	50 µL of Tube 4									
A7, A8			--	50 µL	50 µL of Tube 3									
A9, A10			--	50 µL	50 µL of Tube 2									
A11, A12			--	50 µL	50 µL of Tube 1									
B1, B2			--	50 µL	50 µL of reconstituted std.									
B3, B4			--	50 µL	50 µL of QC1									
B5, B6			--	50 µL	50 µL of QC2									
B7, B8			50 µL	--	50 µL of Sample									
B9, B10			50 µL	--	50 µL of Sample									
B11, B12			50 µL	--	50 µL of Sample									

XI. MICROTITER PLATE ARRANGEMENT

Human PP ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 2	QC2									
B	Blank	Tube 2	QC2									
C	Tube 5	Tube 1	Sample									
D	Tube 5	Tube 1	Sample									
E	Tube 4	Reconstituted Standard	Sample									
F	Tube 4	Reconstituted Standard	Sample									
G	Tube 3	QC 1	Etc.									
H	Tube 3	QC 1	Etc.									

XII. CALCULATIONS

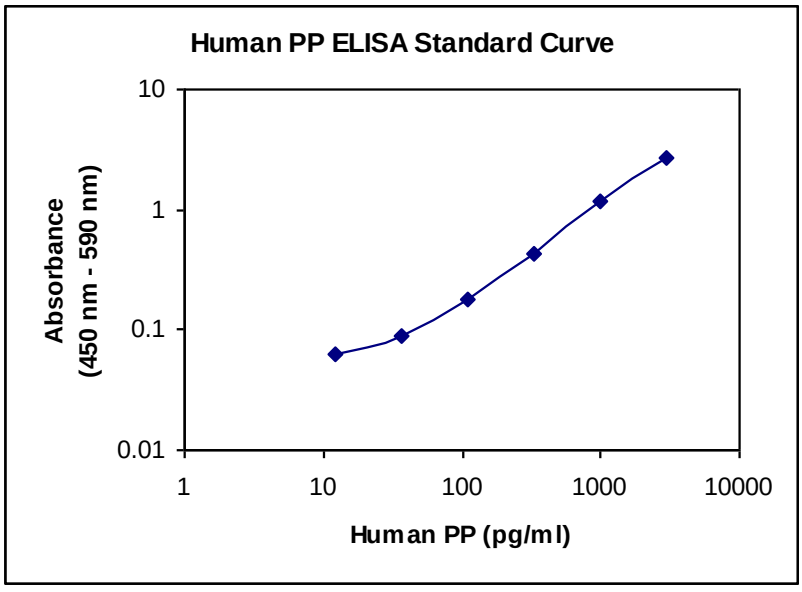
The dose-response curve of this assay fits best to a 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function.

Note: When sample volumes assayed differ from 50 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 25 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50 µL, compensate the volume deficit with matrix solution.

XIII. INTERPRETATION - Acceptance Criteria

1. The assay will be considered accepted when all Quality Control values fall within the calculated Quality Control Range. If any QC's fall outside the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.
3. The limit of sensitivity of this assay is 12.3 pg/mL human PP (50 µL sample size).
4. The appropriate range of this assay is 12.3 pg/mL to 3000 pg/mL human PP (50 µL sample size). Any result greater than 3000 pg/mL in a 50 µL sample should be diluted using matrix solution, and the assay repeated until the results fall within range. Tissue/cell extracts or cell culture media samples greater than 3000 pg/mL in a 50 µL sample should be diluted in Assay Buffer.

XIV. STANDARD CURVE



Typical Standard Curve, not to be used to calculate results.

XV. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of Human PP that can be detected by this assay is 12.3 pg/mL when using a 50 µL sample size.

B. Specificity

The antibody pair used in this assay is specific to Human PP and has no significant cross-reactivity with NPY, PYY, and other gut hormones. This assay does not recognize Rat PP.

C. Precision

Intra-Assay Variation

Sample No.	Mean PP Levels (pg/mL)	Intra-Assay % CV
1	114	3.3%
2	82	4.2%
3	86	4.7%
4	893	5.0%

Inter-Assay Variation

Sample No.	Mean PP Levels (pg/mL)	Inter-Assay % CV
1	103	9.8%
2	74	5.5%
3	812	4.4%
4	128	4.9%

The assay variations of EMD Millipore Human PP ELISA kits were studied on 6 human serum samples with varying concentrations of endogenous PP. The mean intra-assay variation was calculated from results of six duplicate determinations in each assay of the indicated samples. The mean inter-assay variations of each sample was calculated from results of four separate assays with duplicate samples in each assay.

XV. ASSAY CHARACTERISTICS (continued)

D. Spike Recovery of Human PP in Serum

Sample No.	PP Added (pg/mL)	Expected (pg/mL)	Observed (pg/mL)	% of Recovery
1	0	14	14	
	111.1	125.1	107	86%
	333.3	347.3	284	82%
	1000	1014	806	79%
2	0	58	58	
	111.1	169.1	158	93%
	333.3	391.3	370	95%
	1000	1058	1002	95%
3	0	14	2	
	111.1	125.1	108	95%
	333.3	347.3	289	86%
	1000	1014	828	83%

Varying amounts of Human PP were added to three human serum samples and the PP concentration was determined in three separate assays. The % of recovery = observed PP concentrations/expected PP concentrations x 100%.

XV. ASSAY CHARACTERISTICS (continued)

E. Linearity of Serum Dilution

Sample No.	Volume Sampled (µL)	Expected (pg/mL)	Observed (pg/mL)	% Of Expected
1	50	760	760	
	25	380	351	92%
	10	152	148	97%
	5	76	84	111%
2	50	958	958	
	25	479	428	89%
	10	191.6	181	94%
	5	95.8	114	119%
3	50	227	227	
	25	113.5	98	86%
	10	45.4	41	90%
	5	22.7	23	101%

Three human serum samples with the indicated sample volumes were assayed. Required amounts of matrix were added to compensate for lost volumes below 50 µL. The resulting dilution factors of neat, 2, 5, and 10 representing 50 µL, 25 µL, 10 µL, and 5 µL sample volumes assayed, respectively, were applied in the calculation of observed PP concentrations. % expected = observed/expected x 100%.

XVI. QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website emdmillipore.com using the catalog number as the keyword.

XVII. TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 3.0 units or higher after acidification.
9. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with Wash Buffer or 3) overexposure to light after substrate has been added.

XIX. REPLACEMENT REAGENTS

Reagents	**Cat. #**
Human PP ELISA Plate	EP40
10X HRP Wash Buffer Concentrate (50 ml)	EWB-HRP
Human PP Standard	E8040K
Human PP Quality Controls 1 and 2	E6040K
Assay Buffer	EAB-P
Matrix Solution	EMTXPP
Human PP Detection Antibody	E1040
Enzyme Solution	EHRP-3
Substrate	ESS-TMB
Stop Solution	ET-TMB
10-pack of Human Pancreatic Polypeptide (PP) ELISA kits	EZHPP-40BK

XX. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

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

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.