Human PYY (Total)

96-Well Plate

Cat. # EZHPYYT66K

HUMAN PYY (TOTAL) ELISA KIT
96-Well Plate (Cat. # EZHPYYT66K)

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HUMAN PYY (TOTAL) ELISA KIT
96-Well Plate (Cat. # EZHPYYT66K)

I. INTENDED USE

This Human PYY (Total) ELISA kit is used for the non-radioactive quantification of human PYY (Total) in serum and plasma. One kit is sufficient to measure 38 unknown samples in duplicate. PYY is one of the key GI hormones regulating appetite and energy balance in animal. The blood PYY level is low after fasting and elevates significantly after meal. ***This kit is for research purpose only.***

II. PRINCIPLES OF PROCEDURE

This assay is a Sandwich ELISA based on: 1) binding of human PYY molecules (both 1~36 and 3~36) in the sample by rabbit anti-human PYY IgG and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titered amount of anti-rabbit IgG antibodies, 2) and the simultaneous binding of a second biotinylated antibody to the PYY, 3) wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 4) wash away of free enzyme, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetra-methylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm, corrected from the absorbency at 590nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured human PYY (both 1~36 and 3~36) in the unknown sample, the concentration of total PYY can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human PYY.

III. REAGENTS SUPPLIED

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

A. Microtiter Plate
Coated with pretitered antibodies.
Quantity: 1 strip plate
Preparation: Ready to Use
Note: Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8 °C.

B. Adhesive Plate Sealer
Quantity: 2 sheets
Preparation: Ready to Use

III. REAGENTS SUPPLIED (continued)

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 deionized water.

D. Human PYY Standard

Human PYY 3~36 in Assay Buffer: 10, 40, 100, 200, 500, 1000, and 2000 pg/mL.
Quantity: Set of 7 standards, 0.5mL/bottle
Preparation: Ready to Use

E. Human PYY Quality Controls 1 and 2

Purified Recombinant Human PYY 3~36 in Buffer.
Quantity: 0.5mL/bottle
Preparation: Ready to Use

F. Matrix Solution

Serum matrix containing DPP IV inhibitor.
Quantity: 1.5 mL/vial
Preparation: Ready to Use

G. Assay Buffer

0.05 M Borate Saline, pH 8.5, containing 0.025 M EDTA, 0.08% Sodium Azide, 0.1% BSA.
Quantity: 5 mL/vial
Preparation: Ready to Use

H. Human PYY (Total) Capture Antibody

Pre-titered rabbit anti-human PYY antibody
Quantity: 3 mL/vial
Preparation: Mix 1:1with Human PYY Detection Antibody before use, according to § VIII, A.

I. Human PYY Detection Antibody

Pre-titered biotinylated anti-human PYY antibody complementary to capture antibody.
Quantity: 3 mL/vial
Preparation: Mix 1:1with Human PYY (Total) Capture Antibody before use, according to § VIII,A.

J. Blocking Solution

Proprietary reagents to block false positive signals in assay sample.
Quantity: 3 mL/vial
Preparation: Ready to Use.

III. REAGENTS SUPPLIED (continued)

K. Enzyme Solution

Pre-titered Streptavidin-Horseradish Peroxidase Conjugate in Buffer.
Quantity: 12 mL/vial
Preparation: Ready to Use

L. Substrate

3, 3',5,5'-tetramethylbenzidine in Buffer.
Quantity: 12 mL/vial
Preparation: Ready to Use.
Minimize exposure to light.

M. Stop Solution

0.3 M HCl
Quantity: 12 mL/vial
Preparation: Ready to Use
Caution: Corrosive Solution

IV. STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 4 weeks at 2 - 8°C. For longer storage (> 4 weeks), freeze Matrix Solution, Blocking Solution, PYY Standards and Quality Controls at ≤ –20°C. Minimize repeated freeze and thaw of these solutions. 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 has been added to certain reagents as a preservative. Although the concentrations are low, Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. Flush with a large volume of water to prevent azide build-up.

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 eye. Do not swallow or ingest.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and Pipette Tips: 20 µL ~ 100 µL
2. Multi-Channel Pipettes and Pipette Tips: 10 µL ~ 50 µL and 50 µL ~ 300 µL ranges
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. De-ionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm and 590nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth
9. **Optional** DPP IV Inhibitor and Protease Inhibitors, AEBSF or Aprotinin, for blood collection.

VII. SAMPLE COLLECTION AND STORAGE

1. If same blood sample is to be used for both the total PYY and specifically the PYY 3~36 determinations, DPP IV inhibitor (Millipore Cat # DPP4) should be added immediately to the blood after drawing and following vendor instructions.

2. To prepare serum samples, whole blood is directly drawn into a Vacutainer® serum tube that contains no anticoagulant. For long term storage of sample we recommend addition of either AEBSF or aprotinin to a final concentration of 1 mg/mL or 500 KIU/mL, respectively. Mix well and let blood clot at room temperature for 30 min.

3. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at $4 \pm 2^{o}C$.

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

5. Use freshly prepared serum or store samples in aliquots at $\leq -20^{o}C$ for later use. Avoid repeated freeze/thaw cycles.

6. To prepare plasma samples, whole blood should be collected into Vacutainer® EDTA-plasma tubes and placed on ice. For long term storage of sample we recommend addition of either AEBSF or aprotinin to a final concentration of 1 mg/mL or 500 KIU/mL, respectively, mix well and centrifuge at 2,000 to 3,000 x g for 15 min at $4 \pm 2^{o}C$. Observe the same precautions in the preparation of serum samples.

7. Other protease inhibitors or cocktails of inhibitors may be used instead of, but the optimal concentrations to offer protection of PYY should be pre-determined.

8. 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.

9. Avoid using samples with gross hemolysis or lipemia.

VIII. REAGENT PREPARATION

A. Preparation of Capture and Detection Antibody Mixture

Prior to use, measure and combine equal amounts of the Human PYY (Total) Capture Antibody (3mL) and Human PYY Detection Antibody (3mL). Invert to mix thoroughly. If the total volume of antibody mixture needed for the assay is less than 6 mL, mix the two antibody solutions at equal volume and keep the rest separated for next assay. Prepare mixture immediately prior to use. Discard unused remaining mixture after use.

IX. ASSAY PROCEDURE

Pre-warm all reagents to room temperature immediately before setting up the assay. Thaw frozen reagents in luke-warm water if necessary.

1. Dilute the 10X HRP wash buffer concentrate 10 fold by mixing the entire contents of both buffer bottles with 900 mL de-ionized or glass distilled water.

2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C. Assemble the strips in an empty plate holder and fill each well with 300 µL diluted (1X) Wash Buffer. Decant wash buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 3 times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

3. Add 20 µL Matrix Solution to Blank, Standard, and Quality Control wells (refer to X for suggested well orientations).

4. Add 20 µL Assay Buffer to each of the Blank and sample wells.

5. Add in duplicate 20 µL human PYY standards in order of ascending concentration to the appropriate wells.

6. Add in duplicate 20 µL QC1 and 20 µL QC2 to the appropriate wells.

7. Add sequentially 20 µL of the unknown samples in duplicate to the remaining wells**.**

8. Add 20 µL Blocking Solution to each well. Cover the plate with plate sealer and incubate at room temperature for 30 min on an orbital microtiter plate shaker set to rotate at moderate speed (approximately 400 to 500 rpm).

IX. ASSAY PROCEDURE (continued)

9. Remove plate sealer **[CAUTION: Do Not Decant At This Step]** and add 50 µL of the 1:1 mixture of capture and detection antibodies with a multi-channel pipette. Re-cover plate with 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).

10. Remove plate sealer and decant solution from the plate. Tap as before to remove residual solution in the wells. Wash wells 3 times with 1X HRP wash buffer, 300 µl per well per wash. Decant and tap firmly after each wash to remove residual buffer.

11. Add 100 µl Enzyme Solution to each well with a multi-channel pipette. Cover the plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

12. Remove sealer, decant solutions from the plate, and tap plate to remove the residual fluid. Wash wells 6 times with 1X HRP wash buffer, 300 µl per well per wash. Decant and tap firmly after each wash to remove residual buffer.

13. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake in the plate shaker for 5 - 20 minutes. Blue color should be formed in wells of reference standards with intensity proportional to increasing concentrations of PYY.

    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.

    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 into yellow after acidification. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there is no air bubbles in any well.

Assay Procedure for Human (Total) PYY ELISA Kit (Cat. # EZHPYYT66K)

	Step 1	Step 2	Step 3	Step 4	Step 5 - 7	Step 8		Step 9 - 10		Step 11 - 12		Step 13			
Well #	Dilute both bottles of 10X HRP Wash Buffer with 900 mL de-ionized water.	Wash plate 3X with 300 µl diluted HRP wash buffer. Remove residual buffer by tapping smartly on absorbent towels	Matrix Solution	Assay Buffer	Standards/QCs/ Samples	Blocking Solution	Cover plate with sealer. Agitate. Incubate 30 minutes at room tempearature.	Mixture of Capture and Detection Abs	Reseal Plate. Agitate. Incubate 1.5 hours at room temperature. Remove sealer, wash wells, 3X with 300 µl diluted HRP Wash Buffer.	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature. Wash 6X with 300 µl diluted HRP Wash Buffer.	Substrate	Reseal Plate, Agitate, Inbuate 5-20 minutes at room temperature.	Stop Solution	Plate Reader
A1, A2			20 µl	20 µl	--	20 µl		50 µl		100 µl		100 µl		Remove Sealer, Add 100 µl to each well, Mix well. Deflate air-bubbles if necessary	Read Absorbance at 450 nm and 590 nm within 5 min after addition of Stop Solution
B1, B2			20 µl	--	20 µl of 10 pg/mL Standard										
C1, C2			20 µl	--	20 µl of 40 pg/mL Standard										
D1, D2			20 µl	--	20 µl of 100 pg/mL Standard										
E1, E2			20 µl	--	20 µl of 200 pg/mL Standard										
F1, F2			20 µl	--	20 µl of 500 pg/mL Standard										
G1, G2			20 µl	--	20 µl of 1,000 pg/mL Standard										
H1, H2			20 µl	--	20 µl of 2,000 pg/mL Standard										
A3, A4			20 µl	--	20 µl of QC 1										
B3, B4			20 µl	--	20 µl of QC 2										
C3, C4			--	20 µl	20 µl of Sample 1										
D3, D4 Etc.			--	20 µl	20 µl of sample 2										

X. MICROTITER PLATE ARRANGEMENT

A	Blank	Blank	QC 1	QC 1								
B	Standard 1 10 pg/mL	Standard 1 10 pg/mL	QC 2	QC 2								
C	Standard 2 40 pg/mL	Standard 2 40 pg/mL	Sample1	Sample1								
D	Standard 3 100 pg/mL	Standard 3 100 pg/mL	Sample 2	Sample 2								
E	Standard 4 200 pg/mL	Standard 4 200 pg/mL	Etc.	Etc.								
F	Standard 5 500 pg/mL	Standard 5 500 pg/mL										
G	Standard 6 1,000 pg/mL	Standard 6 1,000 pg/mL										
H	Standard 7 2,000 pg/mL	Standard 7 2,000 pg/mL										
	1	2	3	4	5	6	7	8	9	10	11	12

XI. CALCULATIONS

Graph a reference curve by plotting the absorbance unit of 450nm, less unit at 590nm, on the Y-axis against the concentrations of PYY standard on the X-axis The dose-response curve of this assay fits best to a sigmoidal 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 20 µL, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 10 µL of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µL, compensate the volume deficit with either matrix solution or assay buffer, whichever is appropriate.

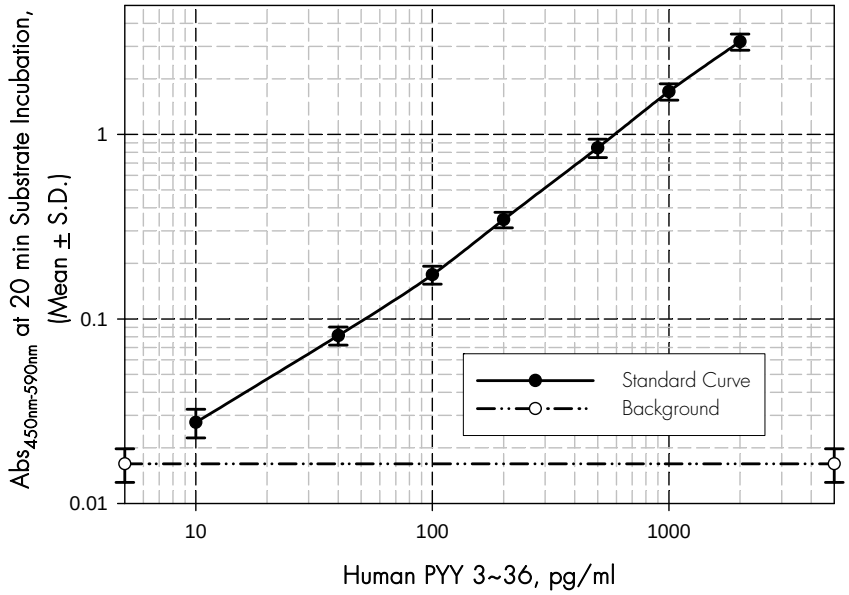
XII. INTERPRETATION

1. The assay should be rejected if one of the two QCs falls outside of 2 standard deviations of the applicable mean. See the supervisor.
2. If the difference between duplicate results of a sample is >15% CV, repeat the sample.
3. The theoretical minimal detecting concentration of this assay is 10 pg/mL human PYY (20 µL sample size).
4. The dynamic range of this assay is 10 pg/mL to 2,000 pg/mL human PYY (20 µL sample size). Any result greater than 2,000 pg/mL in a 20 µL sample should be diluted using matrix solution or assay buffer as diluent, whichever is appropriate, and the assay repeated until the results fall within range.

XIII. **Graph of Typical Reference Curve**

Human PYY (Total) ELISA:

Graph of Typical Standard Curve (n = 15 assays)



For Demonstration Only - Do not use for calculations

XIV. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of PYY (Total) that can be detected by this assay is 10 pg/mL when using a 20 µL sample size.

B. Specificity

The specificity (also known as selectivity) of the analytical test is its ability to selectively measure the analytes in the presence of other like components in the sample matrix.

Human PYY 3~36	100%
Human PYY 1~36	104%
Porcine PYY 3~36	4%
Porcine PYY 1~36	1%

Note: Amino acid sequence of PYY is identical among porcine, canine, rat and mouse.

Human [Leu31,Pro34] PYY	138%
Human [Pro34] PYY	158%
Human and Rat NPY	n.d.
Human and Rat PPP	n.d.
Human Ghrelin	n.d.
Des-Octanoyl Human Ghrelin	n.d.
Human GIP 1~42	n.d.
Human GIP 3~32	n.d.
Glucagon	n.d.
Human GLP-1	n.d.
Human Leptin	n.d.
Human Insulin	n.d.
Human C-peptide	n.d.
Human Amylin	n.d.
Human Adiponectin	n.d.

n.d.: not detectable up to 50 nM concentration

XIV. ASSAY CHARACTERISTICS (continued)

C. Precision

Intra- and Inter-Assay Variations

Sample	PYY (pg/mL) Mean, n = 6	Intra-assay CV (%)	Inter-assay CV (%)
#1, serum	38.9	2.66	6.93
#2, serum	83.2	1.79	6.07
#3, serum	173.2	1.52	6.75
#4, plasma	45.3	5.78	3.65
#5, plasma	115.9	1.00	16.50
#6, plasma	219.9	0.86	4.56

The assay variations of Millipore Human PYY (Total) ELISA kits were studied on three human serum and plasma samples with varying concentrations of endogenous PYY. Intra-assay variations were calculated from results of six duplicate determinations in one assay. Inter-assay variations were calculated from results of six separate assays with duplicate samples in each assay.

XIV. ASSAY CHARACTERISTICS (continued)

D. Spike Recovery Rate of Human PYY in Assay Samples

Sample I.D.	PYY 3~36 Spiked, pg/mL	Serum		Plasma	
		pg/mL	Recovery Rate	pg/mL	Recovery rate
A	0 (Basal)	119	--	122	--
	40	154	88%	163	103%
	100	205	86%	214	92%
	500	598	96%	605	97%
B	0 (Basal)	115	--	130	--
	40	145	75%	171	103%
	100	197	82%	232	102%
	500	525	82%	658	106%
C	0 (Basal)	144	--	172	--
	40	172	70%	214	105%
	100	215	71%	266	94%
	500	558	83%	700	106%
D	0 (Basal)	92	--	104	--
	40	128	90%	145	103%
	100	171	79%	201	97%
	500	511	84%	647	109%
F	0 (Basal)	75	--	78	--
	40	115	100%	114	90%
	100	167	92%	167	89%
	500	566	98%	572	99%
I	0 (Basal)	77	--	81	--
	40	109	80%	124	108%
	100	157	80%	183	102%
	500	490	83%	607	105%
J	0 (Basal)	92	--	99	--
	40	125	83%	137	95%
	100	182	90%	190	91%
	500	557	93%	584	97%
O	0 (Basal)	68	--	72	--
	40	101	83%	110	95%
	100	153	85%	166	94%
	500	513	89%	562	98%
Q	0 (Basal)	151	--	159	--
	40	189	95%	202	108%
	100	227	76%	252	93%
	500	599	90%	656	99%
T	0 (Basal)	136	--	156	--
	40	168	80%	203	118%
	100	228	92%	261	105%
	500	598	92%	662	101%
MEAN ± S.D. (n = 10)	**40**		**84.3 % ± 9.1%**		**102.5 % ± 7.8 %**
	100		**83.3 % ± 7.0 %**		**95.9 % ± 5.4 %**
	500		**88.9 % ± 5.9 %**		**101.6 % ± 4.3 %**

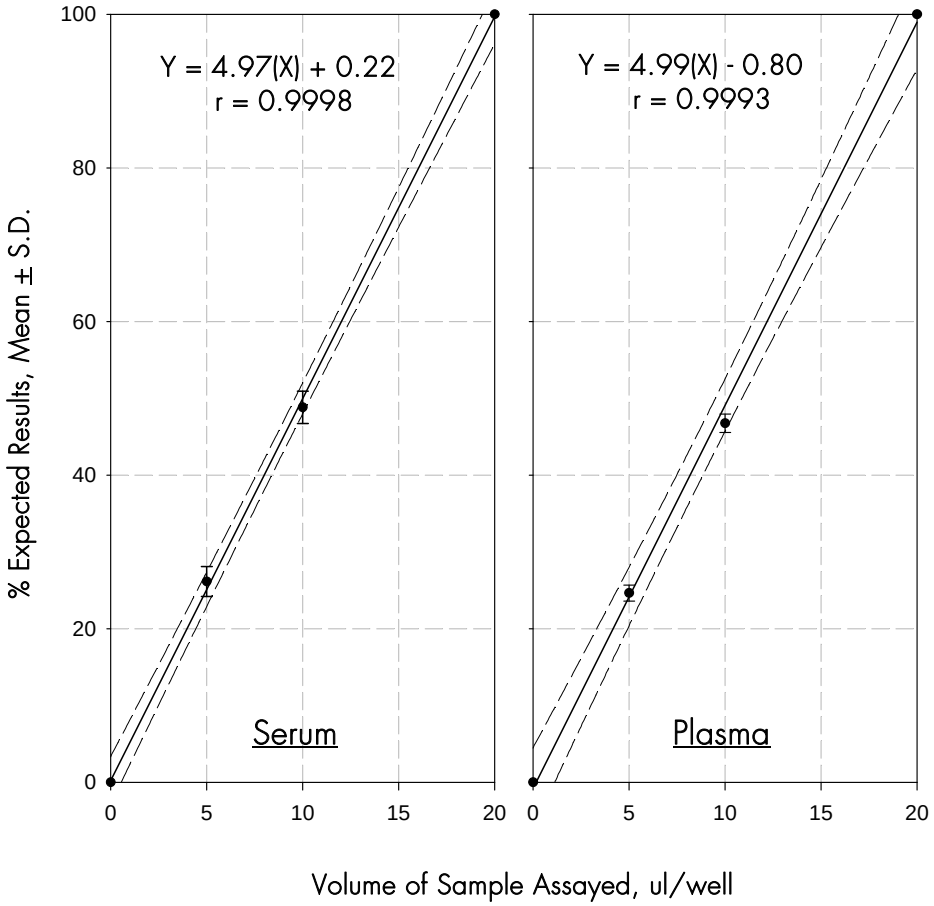
Varying amounts of human PYY 3~36 were added to 10 human serum and plasma samples and total PYY content of each sample was assayed by Human PYY (Total) ELISA. The recovery rate = (observed PYY concentration - Basal PYY concentration) / spiked PYY concentration x 100%.

XIV. ASSAY CHARACTERISTICS (continued)

E. Linearity of Sample Dilution

Human PYY (Total) ELISA:
Sample Dilution Linearity Test

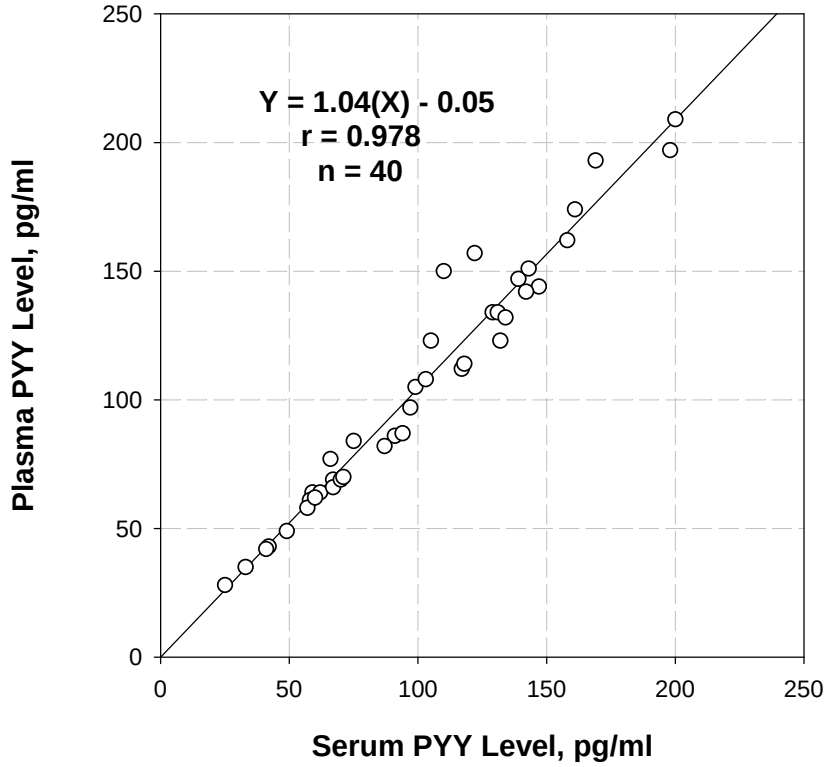
Solid line: Linear Regression Line
Dashed lines: 95% Confidence Interval



Thirteen post-prandial human serum and plasma samples with 80 ~ 220 pg/ml endogenous PYY (Total) are assayed at 20, 10 and 5 ul each for total PYY. The value of each sample obtained from 20 ul is defined as 100% expected.

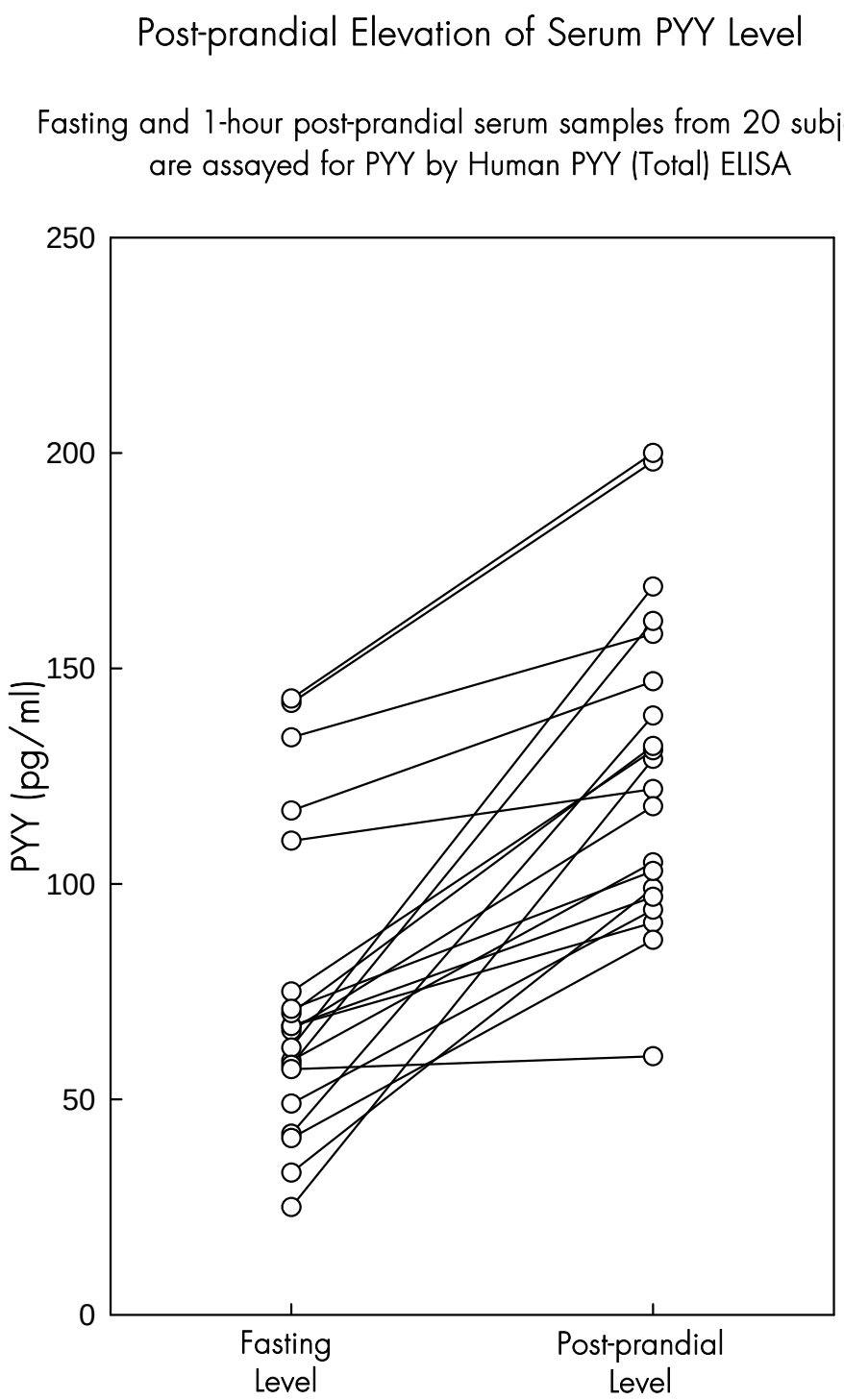
XV. NORMAL RANGE OF PYY (TOTAL) LEVELS IN HUMAN BLOOD

Correlation Between Serum and Plasma PYY Levels



Pre- and post-prandial serum and plasma samples from 20 subjects are assayed by Human PYY (Total) ELISA. The results of serum/plasma pair are analyzed by linear regression analysis.

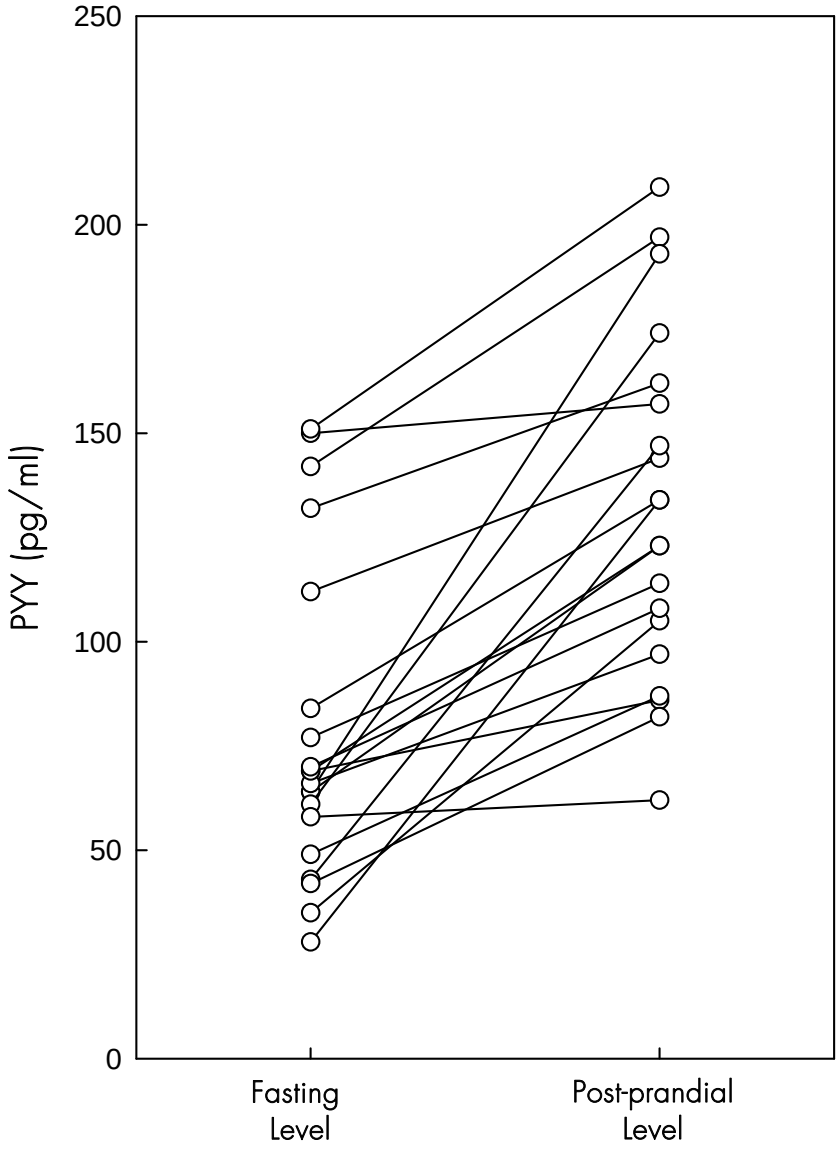
XVI. POST-PRANDIAL ELEVATION OF PYY LEVELS



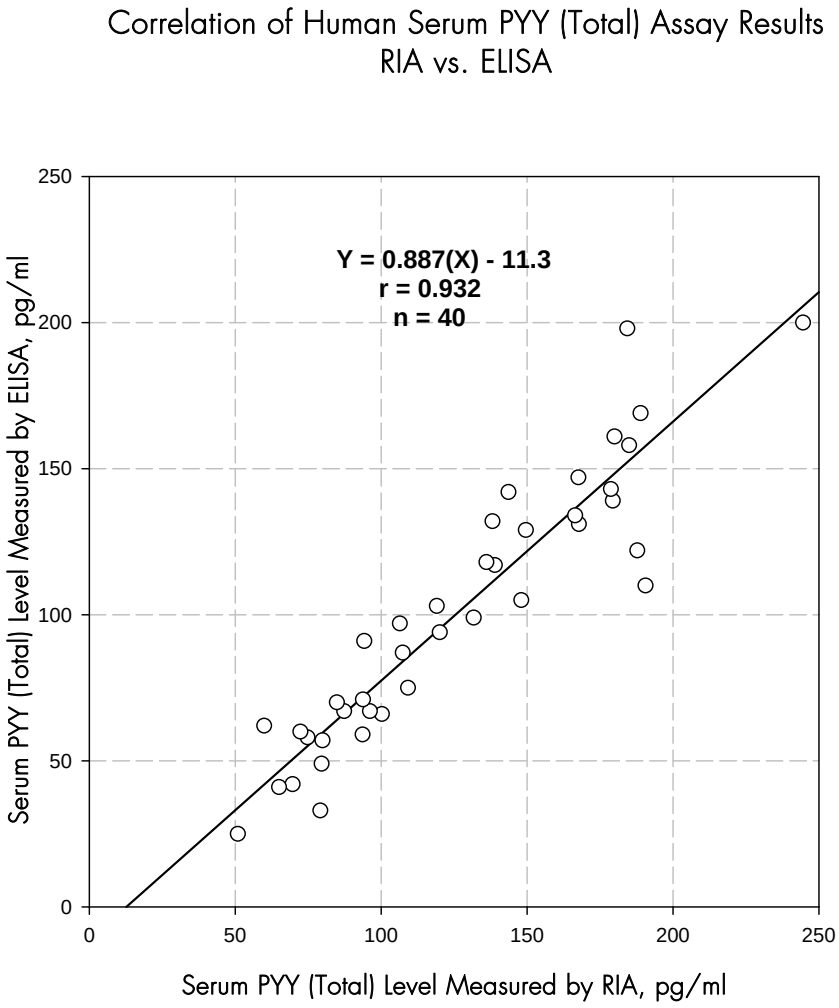
XVI. POST-PRANDIAL ELEVATION OF PYY LEVELS (continued)

Post-prandial Elevation of Plasma PYY Level

Fasting and 1-hour post-prandial plasma samples from 20 subjects are assayed for PYY by Human PYY (Total) ELISA



XVII. CORRELATION GRAPH



Fasting and post-prandial serum samples from 20 normal subjects are assayed for total PYY content by RIA (Cat.#PYYT-66HK) and by ELISA (Cat.#EZPYYT-66K). The paired results from different method are compared by linear regression analysis.

XVIII. 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. 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 HRP Wash Buffer or 3) overexposure to light after substrate has been added.

XIX. REPLACEMENT REAGENTS

Reagents	**Cat. #**
Microtiter Plates	EPDAR
10X HRP Wash Buffer Concentrate	EWB-HRP
Human PYY Standards	E8066-K
Human PYY Quality Controls 1 and 2	E6066-K
Matrix Solution	EMTX-PS
Assay Buffer	EAB
Human PYY (Total) Capture Antibody	E1066-C
Human PYY Detection Antibody	E1066-D
Blocking Solution	EBS
Enzyme Solution	EHRP-3
Substrate	ESS-TMB2
Stop Solution	ET-TMB

XX. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

TELEPHONE ORDERS:
Toll Free US: (800) MILLIPORE

FAX ORDERS: (636) 441-8050

MAIL ORDERS: Millipore
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

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C. Material Safety Data Sheets (MSDS)

Material safety data sheets for Millipore products may be ordered by fax or phone. See Section A above for details on ordering.