User Guide

Human PYY (Total)

96-Well Plate

EZHPYTT66K

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

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.

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Principles of Assay

This assay is a Sandwich ELISA based on:

- 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
- The simultaneous binding of a second biotinylated antibody to the PYY
- Wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies
- Wash away of free enzyme
- 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.

Reagents Supplied

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

Note: Store all reagents at 2-8 °C.

Reagents Supplied	Volume	Quantity	Cat. No.
Microtiter Plate with 2 plate sealers **Note:** Unused strips should be resealed in the foil pouch with desiccant provided and stored at 2-8 °C.	-	1 plate 2 sealers	EPDAR
10X HRP Wash Buffer Concentrate	50 mL	2 bottles	EWB-HRP
Human PYY Standard	0.5 mL/vial Lyophilized	1 vial	E8066-K
Human PYY Quality Controls 1 and 2	0.5 mL/vial Lyophilized	1 vial each	E6066-K

Reagents Supplied	Volume	Quantity	Cat. No.
Assay Buffer	5 mL	2 bottles	EAB
Matrix Solution	1.5 mL	1 vial	EMTX-PS7
Human PYY (Total) Capture Antibody	3 mL	1 bottle	E1066-C
Human PYY (Total) Detection Antibody	3 mL	1 bottle	E1066-D
Enzyme Solution	12 mL	1 bottle	EHRP-3
Blocking solution	3 mL	1 bottle	EBS
Substrate Solution	12 mL	1 bottle	ESS-TMB2
Stop Solution 0.3 M HCl (**Caution:** Corrosive Solution)	12 mL	1 bottle	ET-TMB

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/thaw cycles 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.

Reagent Precautions

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.

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 no swallow or ingest.

Note: See next page for Full Hazardous Labels for components in this kit.

Symbol Definitions

Ingredient	Cat. No.	Full Label	
Human PYY (Total) Capture Antibody	E1066-C		**Danger.** 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 PYY (Total) Detection Antibody	E1066-D		**Danger.** 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 PYY Standard	E8066-K		**Danger.** Harmful if swallowed. Causes serious eye damage. May damage fertility or the unborn child. Toxic to aquatic life with long lasting effects. Obtain special instructions before use. 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. IF exposed or concerned: Get medical advice/ attention.
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.

Ingredient	Cat. No.	Full Label	
Assay Buffer	EAB		**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 PYY Quality Controls 1 & 2	E6066-K		**Danger.** Harmful if swallowed. Causes serious eye damage. May damage fertility or the unborn child. Toxic to aquatic life with long lasting effects. Obtain special instructions before use. 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. IF exposed or concerned: Get medical advice/ attention.
Blocking Solution	EBS		**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.

Materials Required (Not Provided)

- Multi-channel Pipettes and pipette tips: 10 µL-50 µL and 50 µL-300 µL
- Pipettes and pipette tips: 20 µL-100 µL
- Buffer and Reagent Reservoirs
- Vortex Mixer
- Deionized water
- Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm
- Orbital Microtiter Plate Shaker
- Absorbent Paper or Cloth
- **Optional:** DPP IV Inhibitor and Protease Inhibitors, AEBSF or Aprotinin, for blood collection.

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 (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 ±2°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 ≤ –20 °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 ±2°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.

Reagent Preparation

Preparation of Capture and Detection Antibody Mixture

Prior to use, measure and combine equal amounts of the Human PYY (Total) Capture Antibody (3 mL) and Human PYY Detection Antibody (3 mL). 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.

Standard and Quality Controls Preparation

PYY Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the PYY Standard with 0.5 mL distilled or deionized water into the vial to give a concentration prescribed in the analysis sheet. Invert and mix gently, let sit for five minutes or until completely dissolved then mix well.

2. Label six tubes 1, 2, 3, 4, 5, and 6. Add 0.2 mL Assay Buffer to each of the six tubes. Prepare serial dilutions by adding 0.2 mL of the reconstituted standard to tube 1, mix well and transfer 0.2 mL of tube 1 to tube 2, mix well and transfer 0.2 mL of tube 2 to tube 3, mix well and transfer 0.2 mL of tube 3 to tube 4, mix well and transfer 0.2 mL of tube 4 to tube 5, mix well and transfer 0.2 mL of tube 5 to tube 6, 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.

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

Tube #	Standard Concentration (pg/mL)	Volume of Assay Buffer to Add	Volume of Standard to Add
1	X/2	0.2 mL	0.2 mL of reconstituted standard
2	X/4	0.2 mL	0.2 mL of Tube 1
3	X/8	0.2 mL	0.2 mL of Tube 2
4	X/16	0.2 mL	0.2 mL of Tube 3
5	X/32	0.2 mL	0.2 mL of Tube 4
6	X/64	0.2 mL	0.2 mL of Tube 5

PYY Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the PYY 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 five minutes or until completely dissolved then mix well.

Note: For exact concentration of Quality Control 1 and 2, refer to Analysis Sheet. Unused portions of Quality Controls should be stored at ≤ −20 °C. Avoid multiple freeze/thaw cycles.

Assay Procedure

Warm all reagents to room temperature before setting up the assay.

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. 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 Microtiter Plate Arrangement 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).
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.

14. 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. 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.

Assay Procedure for Human (Total) PYY ELISA Kit

	Step 1	Step 2	Step 3	Step 4	Step 5-7	Step 8		Step 9-10		Step 11-12		Step 13		Step 14	
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	Stds./QCs/ Samples	Blocking Solution	Cover plate with sealer. Agitate. Incubate 30 minutes at room temperature.	Mixture of Capt. & Detect. 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, and Incubate 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 Tube 6 Std.										
C1,C2			20 µL	-	20 µL of Tube 5 Std.										
D1,D2			20 µL	-	20 µL of Tube 4 Std.										
E1,E2			20 µL	-	20 µL of Tube 3 Std.										
F1,F2			20 µL	-	20 µL of Tube 2 Std.										
G1,G2			20 µL	-	20 µL of Tube 1 Std.										
H1,H2			20 µL	-	20 µL of Reconst. Std.										
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										

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Microtiter Plate Arrangement

Human (Total) PYY ELISA

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

Calculations

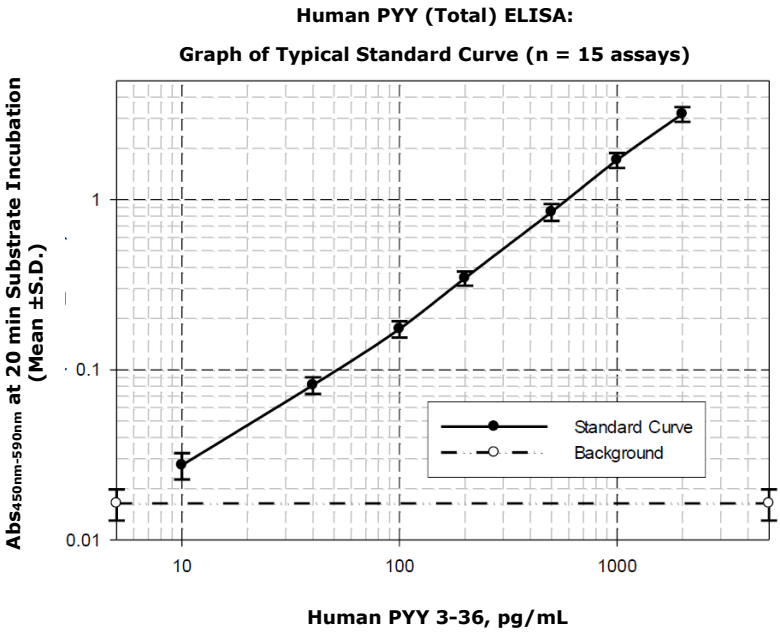
Graph a reference curve by plotting the absorbance unit of 450nm, less unit at 590nm, on the Y-axis against the concentrations of PYY standard on the X-axis The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter Logistic equation. The results of unknown samples can be calculated 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 (for example, 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.

Interpretation

1. The assay will be considered accepted when all Quality Control values fall within the calculated QC range. If any QCs fall outside of the control range, review results with a 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 6.5 pg/mL human PYY (20 µL sample size).
4. The dynamic range of this assay is 14 pg/mL to 1,800 pg/mL human PYY (20 µL sample size). Any result greater than 1,800 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.

Standard Curve



For demonstration only – Do not use for calculations

Assay Characteristics

Sensitivity

The Lowest Level of PYY (Total) that can be detected by this assay is 6.5 pg/mL when using a 20 µL sample size.

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

Precision

Intra- and Inter-Assay Variation

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 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.

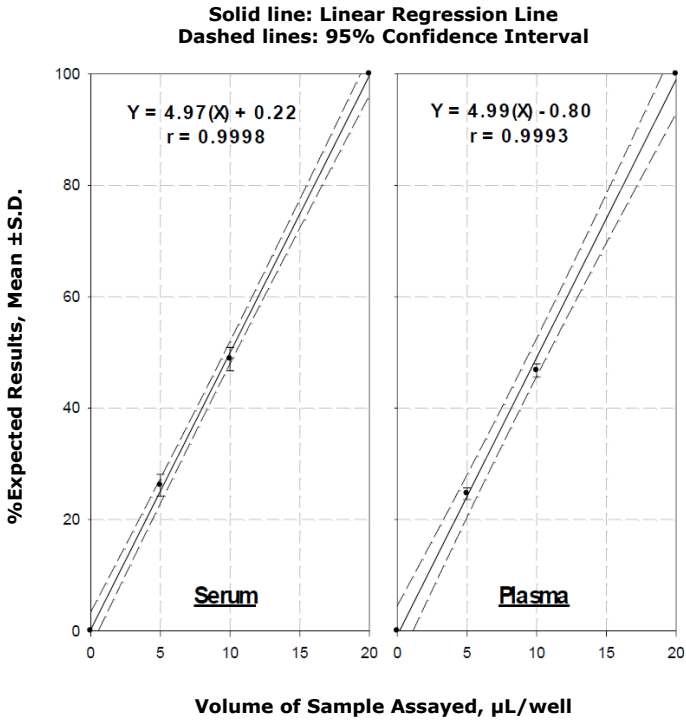
Spike Recovery of Human PYY in Assay Samples

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%.

Linearity of Sample Dilution

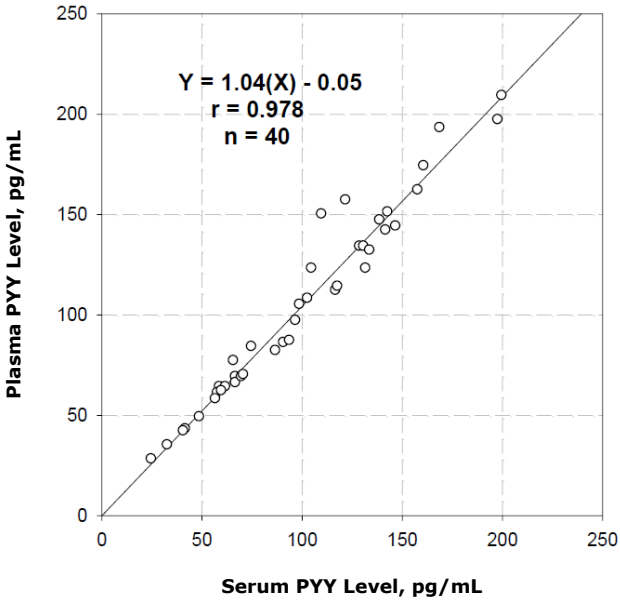
Human PYY (Total) ELISA: Sample Dilution Linearity Test



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.

Normal Range of PYY (Total) Levels in Human Blood

Correlation Between Serum and Plasma PYY Levels

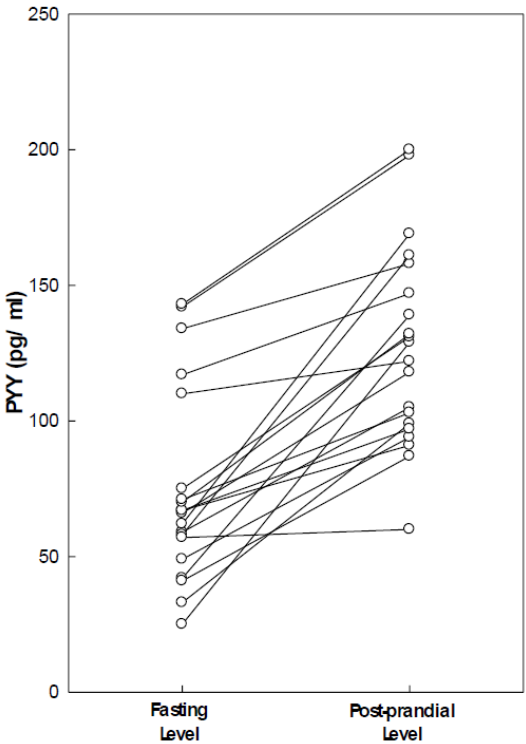


Pre- and post-prandial serum and plasma samples from 20 subjects are assayed by Human PYY (Total) ELISA. The results of serum/plasma pair are analyzed by linear regression analysis.

Post-Prandial Elevation of PYY Levels

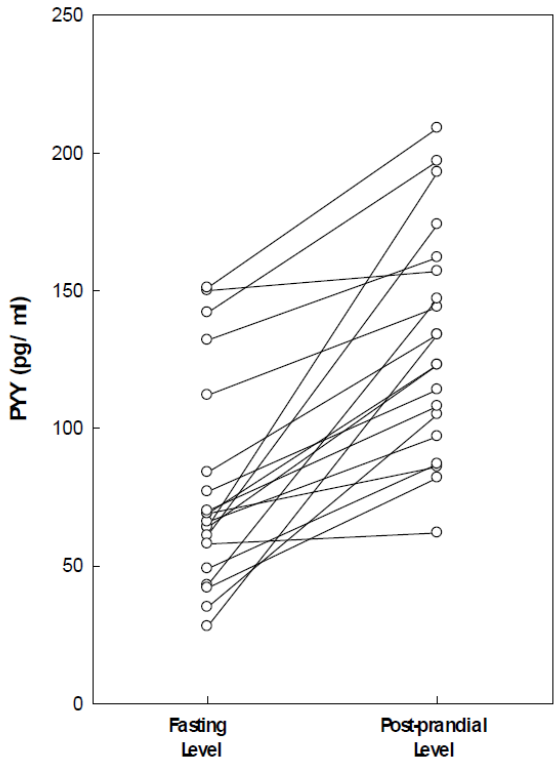
Post-prandial Elevation of Serum PYY Level

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

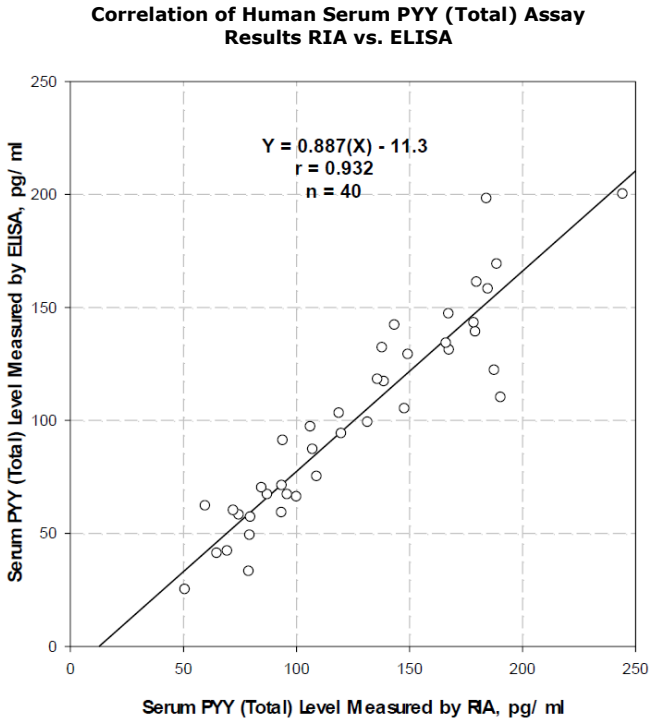


Post-Prandial Elevation of Plasma PYY Level

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



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.

Quality Controls

The ranges for each analyte in Quality Control 1 and 2 are provided on the card insert, or available at our website SigmaAldrich.com.

Troubleshooting

- 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.
- Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
- 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.
- Avoid cross contamination of any reagents or samples to be used in the assay.
- Make sure all reagents and samples are added to the bottom of each well.
- 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.
- Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
- High signal in background or blank wells could be due to:
    - cross well contamination by standard solution or sample, or
    - inadequate washing of wells with Wash Buffer, or
    - overexposure to light after substrate has been added

Product Ordering

Products are available for online ordering at SigmaAldrich.com.

Replacement Reagents

Reagents	Cat. No.
Microtiter ELISA Plate	EPDAR
10X HRP Wash Buffer Concentrate	EWB-HRP
Human PYY Standards	E8066-K
Human PYY Quality Controls 1 and 2	E6066-K
Assay Buffer	EAB
Matrix Solution	EMTX-PS7
Human PYY Detection Antibody	E1066-D
Human PYY Capture Antibody	E1066-C
Blocking Solution	EBS
Enzyme Solution	EHRP-3
Substrate	ESS-TMB2
Stop Solution	ET-TMB
10-pack of Human PYY (Total) ELISA Kits	EZHPYYT-66BK

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