



## **PYY ELISA**

For the quantitative determination of peptide YY in human serum and plasma

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 48-PYYHU-E01.1  
Size: 96 wells  
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### **ALPCO Diagnostics**

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**- Please read the package insert carefully before beginning the assay. -**

## **PYY ELISA**

### **I. Introduction**

This enzyme immunoassay (EIA) kit is a stable and convenient assay system for human peptide YY (PYY). PYY was isolated initially by Tatemoto et al. (1980 ) from the extract of pig duodenum and shown to be a polypeptide consisting of 36 amino acid residues. PYY is homologous to pancreatic polypeptide (PP) and neuropeptide Y (NPY). PYY is localized mainly in endocrine cells in the intestine (ileum, colon, and rectum). PYY shows an inhibitory action on contraction of the gastrointestinal tract and on secretion of pancreatic and gastric juices. PYY is released by food intake. The PYY level in human blood decreases after resection of the intestine, possibly due to the decrease in number of the endocrine cells secreting PYY.

This ELISA kit is prepared by using synthetic human PYY (3-36) as the Standard and biotinylated human PYY (3-36) as the Labeled antigen. The kit can be used for measurement of PYY [both PYY (3-36) and PYY (1-36)] in human serum or plasma with high sensitivity. It will be a specifically useful tool for PYY research.

<b>PYY ELISA Kit</b>	<b>Contents</b>
▼ The assay kit can measure human PYY within the range of 0.082-20 ng/mL.	1) Antibody coated plate
▼ The assay is completed within 16-18 hr. + 3 hr.	2) Standard
▼ With one assay kit, 41 samples can be measured in duplicate.	3) Labeled antigen
▼ Test sample: Human serum and plasma Sample volume: 50 µL	4) SA-HRP solution
▼ The 96-well plate consists of 12 8-well strips, and the strips can be used separately.	5) Enzyme substrate solution (TMB)
▼ Precision and reproducibility Intra-assay CV (%) Human serum 3.67-5.13 Human plasma 6.08-8.52 Inter-assay CV (%) Human serum 2.33-6.55 Human plasma 5.45-10.26	6) Stop solution
▼ Stability and Storage Store all of the components at 2-8°C. The kit is stable under these conditions for 6 months from the date of manufacturing. The expiry date is stated on the package.	7) Buffer solution
	8) Wash solution (concentrated)
	9) Adhesive foil

## II. Characteristics

This EIA kit is used for the quantitative determination of human PYY [both PYY(3-36) and PYY(1-36)] in serum and plasma samples. The kit is characterized by its sensitive quantification and high specificity. In addition, there is no influence by other components of the samples. The human PYY (3-36) Standard is a highly purified synthetic product.

### *Specificity*

The ELISA kit shows 100% cross-reactivity to human PYY (3-36) and human PYY (1-36), and shows less than 0.003% cross-reactivity to human and rat NPY, which have similar amino acid sequences to human PYY.

### *Test Principle*

This ELISA kit for determination of human PYY in samples is based on a competitive enzyme immunoassay using the combination of a highly specific antibody to human PYY and a biotin-avidin affinity system. Standards, samples, and the Labeled antigen are added to the wells of the plate coated with rabbit anti-human PYY antibody for a competitive immunoreaction. After incubation and plate washing, horseradish peroxidase (HRP) labeled streptavidin (SA) is added to form HRP labeled streptavidin-biotinylated antigen-antibody complexes on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of human PYY is calculated.

## III. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	Microplate	1 plate (96 wells)	Rabbit anti-human PYY antibody
2. Human PYY Standard	Lyophilized	1 vial (20 ng)	Synthetic human PYY (3-36)
3. Labeled antigen	Lyophilized	1 vial	Biotinylated human PYY (3-36)
4. SA-HRP solution	Liquid	1 bottle (12 mL)	HRP labeled streptavidin
5. Enzyme substrate solution	Liquid	1 bottle (12 mL)	3, 3',5 ,5'-Tetramethylbenzidine (TMB)
6. Stop solution	Liquid	1 bottle (12 mL)	1M H <sub>2</sub> SO <sub>4</sub>
7. Buffer solution	Liquid	1 bottle (25 mL)	Tris-HCl/saline buffer
8. Wash solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
9. Adhesive foil		3 sheets	

## **IV. Method**

### *Equipment required*

1. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
2. Photometer for microplate (Plate reader), which can read extinction 2.5 at 450 nm
3. Microplate shaker
4. Glass test tubes for preparation of Standard solutions
5. Washing device for microplate and dispenser with aspiration system
6. Graduated cylinder (1,000 mL)
7. Distilled water or deionized water

### *Preparatory work*

1. Preparation of Standard solutions:  
Reconstitute the human PYY Standard with 1 mL of Buffer solution, which yields the 20 ng/mL Standard solution. The reconstituted Standard solution (0.1 mL) is diluted with 0.2 mL of Buffer solution to yield the 6.667 ng/mL Standard solution. Repeat the dilution procedure to make each Standard solution: 2.222, 0.741, 0.247, and 0.082 ng/mL. Buffer solution itself is used as the 0 ng/mL Standard solution.
2. Preparation of Labeled antigen:  
Reconstitute Labeled antigen with 6 mL of Buffer solution.
3. Preparation of Wash solution:  
Dilute 50 mL of Wash solution (concentrated) to 1000 mL with distilled or deionized water.
4. Other reagents are ready for use.

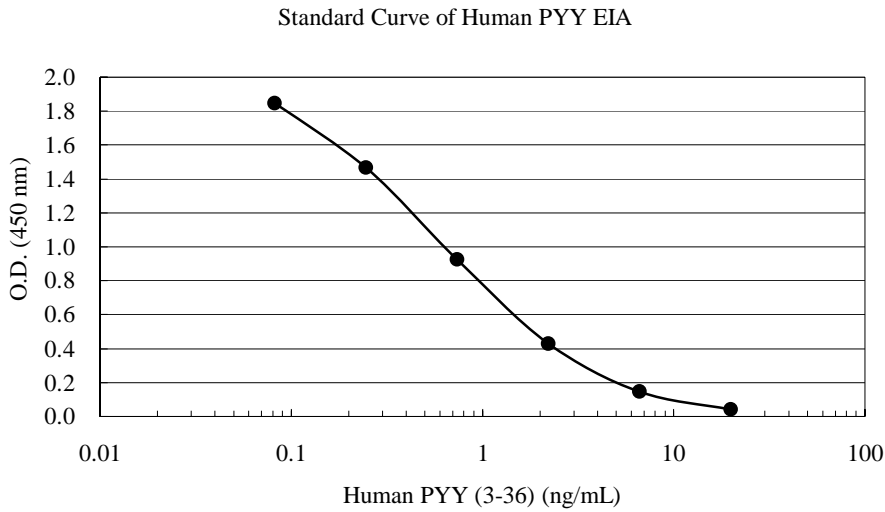
### *Procedure*

1. Before starting the assay, bring all the reagents and samples to room temperature (20-30°C).
2. Add 0.3 mL/well of Wash solution and then aspirate the Wash solution in the wells. Repeat this washing procedure twice (for a total of 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper towels, to ensure most of the residual Wash solution is blotted away.
3. Add 25  $\mu$ L of Buffer solution to the wells first, then introduce 50  $\mu$ L of each of the Standard solutions (0, 0.082, 0.247, 0.741, 2.222, 6.667, and 20 ng/mL) and samples, and finally add 25  $\mu$ L of Labeled antigen to the wells. The total time pipetting the Standard solutions and samples for a whole plate should not exceed 30 minutes.
4. Cover the plate with adhesive foil and incubate it at 4°C overnight for 16 -18 hours. (Plate shaker not needed for this step.)
5. After incubation, move the plate back to room temperature for approximately 40 minutes and take off the adhesive foil. Aspirate and wash the wells 4 times with 0.3 mL/well of Wash solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper towels, to ensure most of the residual Wash solution is blotted away.
6. Pipette 100  $\mu$ L of SA-HRP solution into each of the wells.
7. Cover the plate with adhesive foil and incubate it at room temperature (20 - 30°C) for 2 hours. During the incubation, the plate should be shaken with a plate shaker.
8. Take off the adhesive foil, aspirate, and wash the wells 4 times with 0.3 mL/well of Wash solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper towels, to ensure most of the residual Wash solution is blotted away.
9. Add 100  $\mu$ L of Enzyme substrate solution (TMB) to each of the wells, cover the plate with adhesive foil, and incubate for 30 minutes at room temperature in a dark place for the color reaction. (Plate shaker not needed for this step.)
10. Add 100  $\mu$ L of Stop solution into each of the wells to stop the color reaction.
11. Read the optical absorbance of the solution in the wells at 450 nm. 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. Otherwise, calculate the mean absorbance value of the wells containing Standards and plot a standard curve on semilogarithmic graph paper (abscissa: Standard concentrations; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

## V. Notes

1. If the same blood sample is to be prepared for both the PYY (1-36) and (3-36) determinations (most importantly for the PYY (3-36) determination), DPP IV inhibitor should be added immediately to the serum, plasma, or blood, yielding a final concentration of 100  $\mu$ M.  
EDTA-2Na additive blood collection tubes are recommended for plasma collection. Serum and plasma samples must be used as soon as possible after collection. If the samples are to be tested later, they should be divided into test tubes in small amounts and frozen at or below  $-30^{\circ}\text{C}$ . Avoid repeated freezing and thawing of samples.
2. Standard and Labeled antigen solutions should be prepared immediately before use. The plate strips can be used separately. In such cases, the rest of the reconstituted reagents (Standard and Labeled antigen) should be stored at  $-30^{\circ}\text{C}$  until they are needed for the remaining plate strips.
3. The total pipetting time of Standard solutions and samples, for a whole plate, should not exceed 30 minutes.
4. During storage of the Wash solution (concentrated) at  $2-8^{\circ}\text{C}$ , precipitates may be observed; they will be dissolved when the concentrate is diluted. Diluted Wash solution is stable for 6 months at  $2-8^{\circ}\text{C}$ .
5. Pipetting operations may affect the precision of the assay, pipette Standard solutions or samples precisely into each well of the plate. In addition, use clean test tubes or vessels in the assay and use new tips for each Standard or sample to avoid cross contamination.
6. When a sample's value exceeds 20 ng/mL, the sample needs to be diluted with Buffer solution to a proper concentration within the range of the assay.
7. Perform all of the determinations in duplicate.
8. Read the plate's optical absorbance as soon as possible after the addition of the Stop solution.
9. To quantitate accurately, always run a standard curve when measuring samples.
10. Protect reagents from strong light (e.g., direct sunlight) during storage and assay.
11. Satisfactory performance of the test is guaranteed only when reagents are used from combination packs with identical lot numbers.

## VI. Performance Characteristics



### Analytical recovery

#### *Human Serum A*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.376		
0.2	0.579	0.576	100.52
1.0	1.357	1.376	98.62
5.0	4.712	5.376	87.65

#### *Human Serum B*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.337		
0.2	0.516	0.537	96.09
1.0	1.296	1.337	96.93
5.0	4.897	5.337	91.76

#### *Human Serum C*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.677		
0.2	0.913	0.877	104.10
1.0	1.821	1.677	108.59
5.0	6.257	5.677	110.22

*Human Serum D*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.336		
0.2	0.536	0.536	100.00
1.0	1.307	1.336	97.83
5.0	4.251	5.336	79.67

*Human Plasma A*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.341		
0.2	0.546	0.541	100.92
1.0	1.318	1.341	98.28
5.0	4.447	5.341	83.26

*Human Plasma B*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.336		
0.2	0.548	0.536	102.24
1.0	1.304	1.336	97.60
5.0	4.212	5.336	78.94

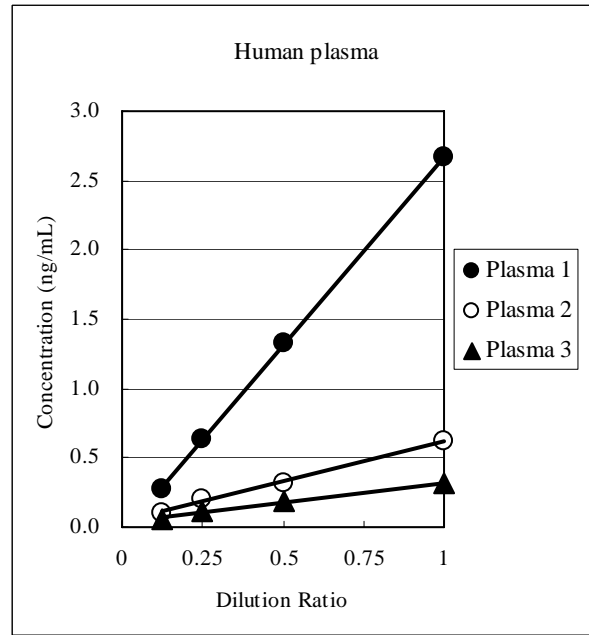
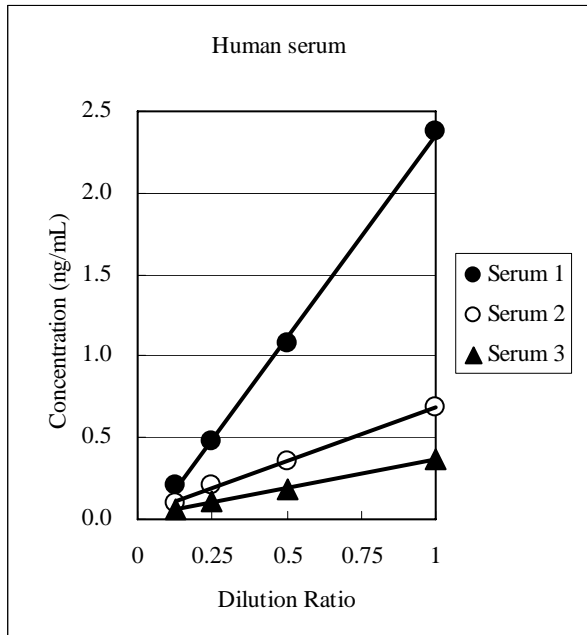
*Human Plasma C*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.605		
0.2	0.847	0.805	105.22
1.0	1.728	1.605	107.66
5.0	5.669	5.605	101.14

*Human Plasma D*

Human PYY added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.331		
0.2	0.538	0.531	101.32
1.0	1.395	1.331	104.81
5.0	4.631	5.331	86.87

*Dilution test*



*Cross-reactivity*

Related peptides	Cross-reactivity (%)
Human PYY(3-36)	100
Human PYY(1-36)	100
Rat/human NPY	< 0.003

*Precision and reproducibility*

Test sample	Intra-assay CV(%)	Inter-assay CV(%)
Human serum	3.67-5.13	2.33-6.55
Human plasma	6.08-8.52	5.45-10.26

**VII. Stability and Storage**

*Storage* Store all of the components at 2-8°C.

*Shelf life* This kit is stable under the above conditions for 6 months from the date of manufacturing. The expiry date is stated on the package.

*Package* For 96 tests per one kit, including Standards

## VIII. References

1. Adrian, T.E., Smith, H.A., Calvert, S.A., Aynsley-Green, A. and Bloom, S.R. (1986): Elevated plasma peptide YY in human neonates and infants. *Pediatr Res.*, **20**:1225-1227.
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5. Greeley, G.H.Jr., Hill, F. L., Spannagel, A.and Thompson, J.C.(1987): Distribution of peptide YY in gastrointestinal tract of the rat, dog, and monkey. *Regul.Pept.*, **19**, 365-372.
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7. Larhammar, D.(1996): Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide: *Regul.Pept.*, **62**, 1-11.