Version 22-04/24

User's Manual

Human PYY ELISA Kit

REF **DEIA4772**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This ELISA kit is used for quantitative determination of human PYY [both PYY(3-36) and PYY(1-36)] in serum and plasma samples.

General Description

This ELISA kit is a stable and convenient assay system for human peptide YY (PYY). PYY was isolated initially by Tatemoto et al. from the extract of pig duodenum and shown to be a polypeptide consisting of 36 amino acids 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 juice. PYY is released by taking diet. The PYY level in human blood decreases after resection of the intestine, possibly be due to the decrease in number of the endocrine cells secreting PYY.

The ELISA kit is prepared by using synthetic human PYY (3-36) as standard and biotinylated human PYY (3-36) as 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.

Principles of Testing

This ELISA kit for determination of human PYY in samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to human PYY and biotin-avidin affinity system. To the wells of plate coated with rabbit anti human PYY antibody, standard or samples, labeled antigen are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled streptoavidin-biotinylated antigen-antibody complex 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.

Reagents And Materials Provided

1. Antibody coated plate, Microtiter plate, 1 plate (96 wells). Main Ingredient: Rabbit anti human PYY antibody
2. Standard, Lyophilized, 1 vial (20 ng). Main Ingredient: Synthetic human PYY (3-36)
3. Labeled antigen, Lyophilized, 1 vial. Main Ingredient: Biotinylated human PYY (3-36)
4. SA-HRP solution, Liquid, 1 bottle (12mL). Main Ingredient: HRP labeled streptoavidin
5. Enzyme substrate, solution, Liquid, 1 bottle (12 mL). Main Ingredient: 3,3', 5,5'-Tetramethylbenzidine (TMB)
6. Stopping solution, Liquid, 1 bottle (12 mL). Main Ingredient: 1M H_2SO_4
7. Buffer solution, Liquid, 1 bottle (25 mL). Main Ingredient: Tris-HCl/saline buffer
8. Washing solution (concentrated), Liquid, 1 bottle (50 mL). Main Ingredient: Concentrated saline
9. Adhesive foil, 3 sheets

Materials Required But Not Supplied

1. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
2. Photometer for microtiter plate (Plate reader), which can read extinction 2.5 at 450 nm
3. Microtiter plate shaker
4. Glass test tubes for preparation of standard solution
5. Washing device for microtiter plate and dispenser with aspiration system
6. Graduated cylinder (1000 mL)
7. Distilled water or deionized water

Storage

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

Reagent Preparation

1. **Preparation of standard solution:**

Reconstitute Standard with 1 mL of buffer solution, which affords 20ng/mL standard solution. The reconstituted standard solution (0.1mL) is diluted with 0.2 mL of buffer solution that yields 6.667 ng/mL standard solution. Repeat the dilution procedure to make each standard solution of 2.222, 0.741, 0.247 and 0.082 ng/mL. Buffer solution itself is used as 0ng/mL.

2. **Preparation of labeled antigen solution:**

Reconstitute Labeled antigen with 6mL of Buffer solution.

3. **Preparation of washing solution:**

Dilute 50 mL of Washing solution (concentrated) to 1000 mL with distilled or deionized water.

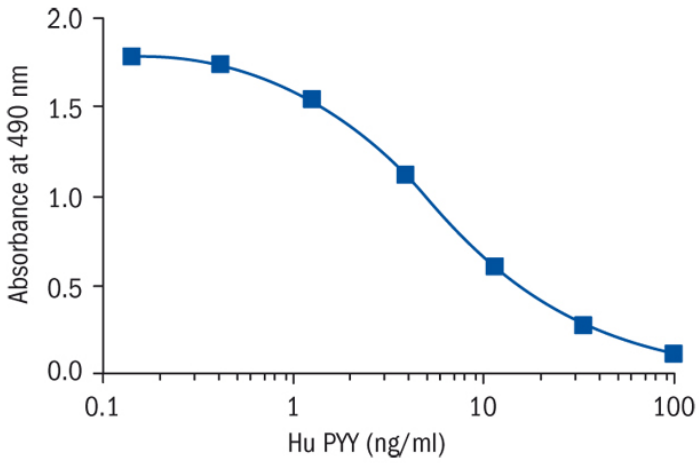
4. **Other reagents are ready for use.**

Assay Procedure

1. Before start assay, bring all the reagents and samples to room temperature (20-30°C).
2. Add 0.3 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Fill 25 µL of buffer solution into the wells first, then introduce 50 µL of each of standard solutions (0, 0.082, 0.247, 0.741, 2.222, 6.667, 20 ng/mL) or samples and finally add 25 µL of labeled antigen into the wells. The total pipetting time of standard solutions and samples for a whole plate should not exceed 30 min.
4. Cover the plate with adhesive foil and incubate it at 4°C overnight for 16-18 hours. (Still, plate shaker not need.)
5. After incubation, move the plate back to room temperature keeping for about 40 minutes and take off the adhesive foil, aspirate and wash the wells 4 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.

6. Pipette 100 µL 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 hour. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).
8. Take off the adhesive foil, aspirate and wash the wells 4 times with approximately 0.3 mL/well of washing solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
9. Add 100 µL of enzyme substrate solution (TMB) to each of the well, cover the plate with adhesive foil and keep it for 30 minutes at room temperature in a dark place for color reaction. (Still, plate shaker not need.)
10. Add 100 µL of stopping solution into each of the wells to stop 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 mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

Typical Standard Curve



Detection Range

0.082-20 ng/ml

Specificity

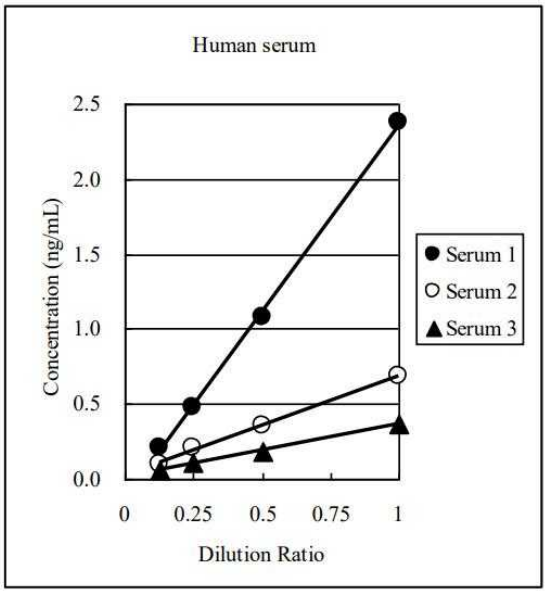
The ELISA kit shows 100% cross reactivity to human PYY (3-36) and human PYY (1-36), and shows less than 0.003% cross reactivity to human and rat NPY, which have similar amino acid sequence with human PYY.

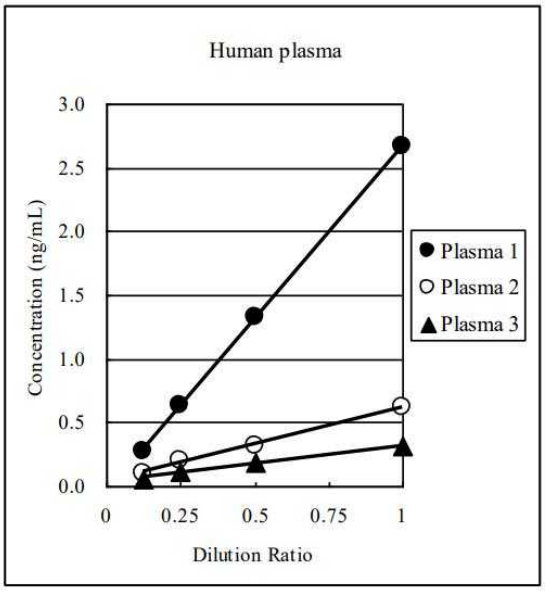
Cross-reactivity

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

Linearity

Dilution test





Recovery

1. 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

2. 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

3. 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

4. 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

5. 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

6. 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

7. 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

8. 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

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

Precautions

1. If same blood sample is to be prepared for measuring PYY(3-36) only using another kit (this kit can measure both of PYY (1-36) and PYY(3-36)), DPP IV inhibitor should be added immediately to the serum, plasma or blood, yielding 100 μM final concentration. EDTA-2Na additive blood collection tube is recommended for the plasma collection. Serum and plasma samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30°C. Avoid repeated freezing and thawing of samples.
2. Standard and labeled antigen solutions should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (standard and labeled antigen) should be stored at -30°C.
3. The total pipetting time of standard solutions and samples for a whole plate should not exceed 30 min.
4. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8°C.
5. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples precisely into each well of plate. In addition, using clean test tubes or vessels in assay and use new tip for each standard or sample to avoid cross contamination.
6. When sample value exceeds 20 ng/mL, it needs to be diluted with buffer solution to proper concentration.
7. Perform all the determination in duplicate.
8. Read plate optical absorbance of reaction solution in wells as soon as possible after stop color reaction.
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 pack with identical lot number.