KAMIYA BIOMEDICAL COMPANY

Human Peptide YY EIA

For the quantitative determination of Peptide YY in human serum and plasma.

Cat. No. KT-378

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The Human Peptide YY EIA is for the quantitative determination of Peptide YY (PYY) in human serum and plasma. For research use only.

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 juice. PYY is released during dieting. 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. The EIA kit is prepared by using a synthetic human PYY (3-36) as calibrator 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. The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influence by other components in samples. Human PYY (3-36) calibrator is highly purified synthetic product.

PRINCIPLE
This EIA 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, calibrators 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 on the wells. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of human PYY is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	Microtiter plate	1 plate (96 wells)	Rabbit anti-human PYY antibody
2. Calibrator	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 streptoavidin
5. Enzyme substrate solution (TMB)	Liquid	1 bottle (12 mL)	3,3',5,5'-Tetramethylbenzidine (TMB)
6. Stopping solution	Liquid	1 bottle (12 mL)	1M H_2SO_4
7. Buffer solution	Liquid	1 bottle (25 mL)	Tris-HCl/saline buffer
8. Washing solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
9. Plate Seal		3 sheets	

MATERIALS REQUIRED BUT NOT PROVIDED
- Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
- Microtiter plate shaker
- Washing device for microtiter plate and dispenser with aspiration system
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- Glass test tubes for preparation of calibrator solution

- Graduated cylinder (1,000 mL)
- Distilled water or de-ionized water

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette calibrator solutions or samples into each well of the plate. In addition, use clean test tubes or vessels in assay and use new tip for each sample or calibrator to avoid cross-contamination

To quantitate accurately, always run a calibration curve when measuring samples.

Read plate optical absorbance of reaction solution in wells as soon as possible after stop color reaction.

When sample value exceeds 20 ng/mL, it needs to be diluted with buffer solution to proper concentration.

Perform all the determination in duplicate.

During storage of the washing solution (concentrated) at 4°C, precipitates may be observed, however, they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 4°C.

The total pipetting time of calibrator solutions and samples for a whole plate should not exceed 30 min.

Calibrator 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 (calibrator and labeled antigen) should be stored at -30℃.

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. 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℃. Avoid repeated freezing and thawing of samples.

REAGENT PREPARATION

1. Preparation of calibrator solution: Reconstitute Calibrator with 1 mL of buffer Solution, which affords 20 ng/mL calibrator solution. The reconstituted calibrator solution (0.1 mL) is diluted with 0.2 mL of buffer solution that yields 6.667ng/mL calibrator solution. Repeat the dilution procedure to make each calibrator solution of 2.222, 0.741, 0.247and 0.082 ng/mL. Buffer solution itself is used as 0 ng/mL.
2. Preparation of labeled antigen solution: Reconstitute Labeled antigen with 6 mL of Buffer solution.
3. Preparation of washing solution: Dilute 50 mL of Washing solution (concentrated) to 1,000 mL with distilled or de-ionized water.
4. Other reagents are ready for use.

STORAGE

Store kit at 4°C.

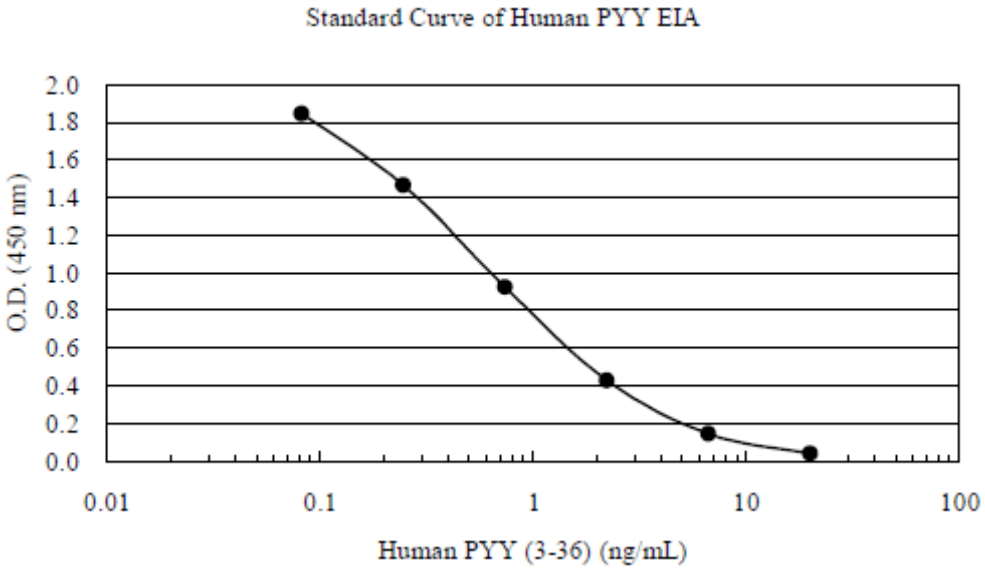
ASSAY PROTOCOL

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 calibrator 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.
4. Cover the plate with Plate Seal and incubate it at 4℃ overnight for 16-18 hours (Still, plate shaker not needed).
5. After incubation, move the plate back to room temperature keeping for about 40 minutes and take off the Plate Seal, 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 Plate Seal 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 Plate Seal, 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 Plate Seal and keep it for 30 minutes at room temperature in a dark place for color reaction. (Still, plate shaker not needed.)
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 calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of calibrator; ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation for this calibration curve.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Analytical Recovery

<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

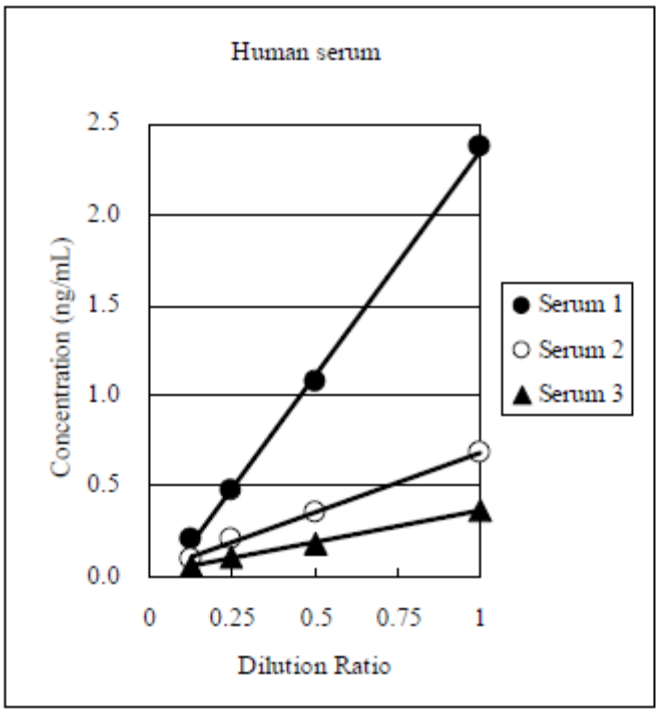
<Human Plasma C>

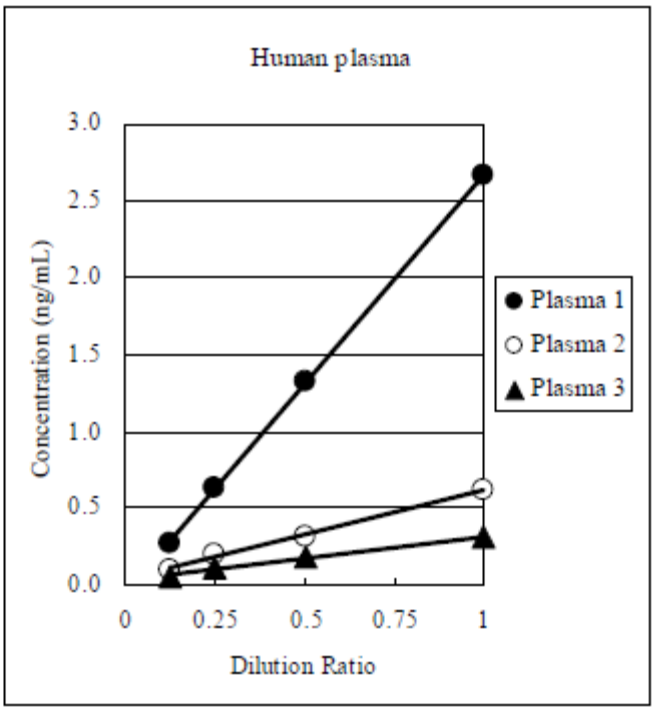
Human PYY added(ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0	0.605		
0.2	0.847	0.805	105.22
1.0	1.728	1.605	107.66
5.0	5.669	5.605	101.14

<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	Crossreactivity (%)
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

FOR RESEARCH USE ONLY

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