YK081 Rat PYY EIA kit

I. Introduction

This enzyme immunoassay(EIA)kit is a stable and convenient assay system for 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 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 decreases after resection of the intestine, possiblly be due to the decrease in number of the endocrine cells secreting PYY.

The EIA kit is prepared by using synthetic rat PYY as standard antigen and biotinylated rat PYY as labeled antigen. The kit contains specific polyclonal antibody recognized to the amino acid sequence of rat PYY.

II. Characteristics

This ELISA kit is used for quantitative determination of rat PYY in rat plasma sample. It has a lot of advantage to perform the assay, such as good quantification, no influence with other body fluid factors or physiogical active substances and needlessness of sample pretreatment. PYY standard is highly purified synthetic product (purity: higher than 98%) and biotinylated peptide is stable because N –biotinylglycylglycyl rat PYY is used for it.

< Specificity>

The EIA kit shows 10% cross reactivity to human PYY and less than 0.01% cross reactivity to human and rat NPY which have similar amino acid sequence with rat PYY.

< Test Principle>

This EIA kit for determination of rat PYY in plasma sample is based on a competitive enzyme immunoassay using combination with highly specific antibody to rat PYY and biotin – avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG and PYY standard or samples, biotinylated rat PYY and anti rat PYY antibody are added to the wells for competitive immunoreaction. After rinsing out excess rat PYY, HRP labeled streptoavidins are added to bind to the antigen-antibody complex so that HRP labeled streptoavidin - biotinylated rat PYY – antibody complexs are formed on the surface on the wells. Finally, excess HRP labeled streptoavidins are rinsed out and HRP enzyme activity is determined and the concentration of rat PYY is calculated.

III. Composition

Component	Form	Quantity	Main Ingredient
1.Antibody coated plate	MTP*1	1 plate(96 wells)	Anti rabbit IgG
2.PYY standard	lyophilized	1 vial	Synthetic rat PYY(100ng)
3.Labeled antigen	lyophilized	1 vial	Biotinylated rat PYY(1.5 ng)
4.PYY antibody	lyophilized	1 vial	Rabbit anti rat PYY
5.SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled streptoavidin
6.Substrate buffer	liquid	1 bottle (12 mL)	0.015% Hydrogen Peroxide
7.OPD tablet	tablet	2 tablets	o-Phenylenediamine hydrochloride
8.Stopping solution	liquid	1 bottle (12 mL)	2N-H_2SO_4
9.Buffer solution (concentrated)	Liquid	1 bottle (12 mL)	Phosphate buffer
10.Washing solution (concentrated)	Liquid	1 bottle (50 mL)	Concentrated saline
11. Adhesive foil		3 sheets	

MTP*1……Microtitration plate

VI. Method

< Equipment required>

1)Photometer for microtitration plate(Plate reader),which can read extinction 2.5 at 490 nm

2)Rotator for microtitration plate

3)Washing device for microtitration plate and dispenser for approximately 0.3 mL with aspiration system

4)Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips

5)Test tubes for preparation of standard solution

6)Graduated cylinder(1,000 mL)

7)Distilled water or deionized water

< Preparatory work>

1)Specimens
Suitable assay specimens are plasma samples(add 1mg EDTA to 1mL blood sample), as fresh as possible or –30°C frozen after dividing into tube with small amount.
100 μL is sufficient amount for the determination.

2)Preparation of buffered solution:
10 mL of buffer solution (concentrated) is to be diluted with 30mL of distilled water, which makes 40mL of diluted buffer solution.

3)Preparation of standard solution:
Reconstitute the standard(lyophilized rat PYY 100ng/vial) with 1mL of diluted buffer solution, which affords 100 ng/mL standard solution. 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of diluted buffered solution, that yields 33.33 ng/mL standard solution. 0.1mL of 33.33 ng/mL standard solution is diluted with 0.2 mL of the diluted buffered solution, that makes 11.11 ng/mL standard solution. Repeat the dilution to make each standard of 3.70, 1.23, 0.41, 0.14, 0.05 ng/mL. Diluted buffered solution is used as 0 ng/mL.

4) Preparation of labeled antigen:
Reconstitute labeled antigen with 6mL of distilled water.

5)Preparation of PYY antibody:
Reconstitute PYY antibody with 12mL of distilled water.

6)Preparation of substrate solution:
Resolve OPD tablet with 12 mL of substrate buffer. It should be prepared immediately before use.

7) Preparation of washing solution:
Dilute 50 mL of washing solution (concentrated) to 1000 mL with distilled or deionized water.

8) Other reagents are ready for use.

< Procedure>

1.Warm up the reagents and samples to room temperature before beginning the test.

2.Add 350μL/well of washing solution into the wells and aspirate the washing solution from the wells. Repeat this washing procedure further twice(total 3 times washing).

3.Fill 50μL of buffered solution into wells first, then introduce 25μL each of standard solutions (0, 0.05, 0.14, 0.41, 1.23, 3.70, 11.11, 33.33 ng/mL) or samples, then add 50μL of labeled antigen and finally introduce 100μL of PYY antibody into the wells .

4.Cover the plate with adhesive foil and incubate it at room temperature (20 ~ 30°C) for 16 - 20 hours.
During the incubation, the plate should be rotated with a plate rotator.

5.Take off the adhesive foil, aspirate the solution in the wells and wash the wells three times with approximately 0.35 mL/well of washing solution.

6.Pipette 100μL of SA-HRP solution into 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 rotated with a plate rotator.

8.Take off the adhesive foil, aspirate and wash the wells four times with approximately 0.3 5mL/well of washing solution.

9.Add 100μL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 30 minutes at room temperature.

10.Add 100μL of stopping solution into the wells to stop reaction.

11.Read the optical absorbance of the wells at 490nm.

12.Calculate mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values.).
13.Use the standard curve to read PYY concentrations in samples from the corresponding absorbance values.

V. Notes

1.Plasma samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amount and frozen at or below – 30°C. Avoid repeated freezing and thawing of plasma samples.

2.PYY standard, labeled antigen, PYY antibody, OPD solution should be prepared immediately before use in assay using clean test tubes or vessels. Diluted washing solution is stable for 6 months at 2 to 8°C.

3.During storage of washing solution (concentrated) at 2 to 8°C, precipitates may be observed, however they will be dissolved when diluted.

4.As pipetting operations may affect with the precision of the assay, pipette precisely standard solutions or samples into each well of plate. And use new tip for each sample to avoid cross contamination.

5.When sample value exceeds 30 ng/mL, it needs to be diluted with buffered solution within the assay range.

6.During incubation except color reaction, the test plate should be rotated gently by plate rotator to promote immunoreaction.

7.During continuous rotation of test plate, the plate rotator may be heated up. It is recommended to place styrene form or plywood between the plate and the rotator.

8.Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction.

9. Perform all the determination in duplicate.

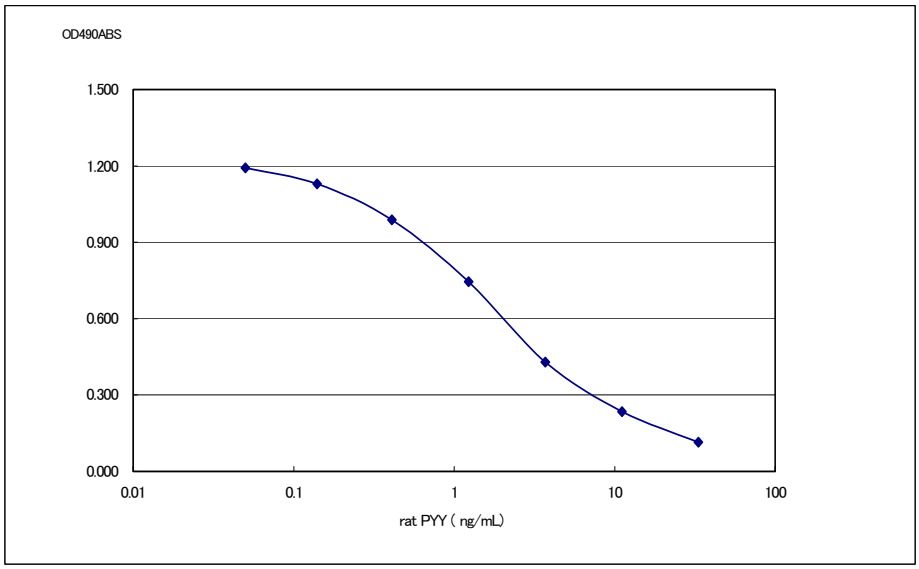
10.To quantitate accurately, always run a standard curve when testing samples.

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

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

VI. Performance Characteristics

Typical standard curve



Analytical recovery

< Rat plasma >

Rat PYY added ng/mL	Observed ng/mL	Expected ng/mL	Recovery %
0.00	1.09		
0.25	1.30	1.34	97.01
1.00	2.42	2.09	115.79
4.00	5.51	5.09	108.25

Precision and reproducibility

- Intra-assay CV(%) 7.95 ~ 12.81
- Inter-assay CV(%) 11.95 ~ 13.61

Assay range

0.05 - 30 ng/mL

VII. Stability and Storage

< Storage > Store all of the components at 2 to 8°C.

< Shelf life > 6 month from the date of manufacturing
The expiry date is described on the label of kit.

< Package > For 96 tests per 1 kit including standards

VIII. References

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5. Greeley, G.H.Jr., Hill, F. L. C., Spannagel, A.and Tompson, 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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