KAMIYA BIOMEDICAL COMPANY

Rat Peptide YY EIA

For the quantitative determination of Peptide YY in rat plasma.

Cat. No. KT-370

For Research Use Only.

PRODUCT INFORMATION

Rat Peptide YY EIA
Cat. No. KT-370

INTENDED USE
The Rat Peptide YY EIA is for the quantitative determination of Peptide YY (PYY) in rat plasma. For research use only.

INTRODUCTION
This enzyme immunoassay (EIA) kit is a stable and convenient assay system for rat 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 decreases after resection of the intestine, possibly due to the decrease in number of the endocrine cells secreting PYY. This kit is prepared by using a synthetic rat PYY as the calibrator and biotinylated rat PYY as the labeled antigen. The kit contains a specific polyclonal antibody that recognizes the amino acid sequence of rat PYY. The advantages of this kit include high sensitivity, high specificity, no interference from other plasma components, and no sample pre-treatment. The PYY Calibrator is a highly purified synthetic product (purity: > 98%).

PRINCIPLE
This EIA kit is based on a competitive enzyme immunoassay using a highly specific antibody combined with a biotin-avidin affinity system. The 96-well plate is coated with goat anti-rabbit IgG and PYY Calibrators or samples, labeled antigen and anti-rat PYY antibodies are added to the wells for a competitive immuno-reaction. After incubation and washing, HRP-labeled streptoavidins are added to form HRP-labeled streptoavidin-biotinylated rat PYY-antibody complexes on the surface of the wells. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) and the concentration of rat PYY is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Goat anti-Rabbit IgG
2. PYY Calibrator	Lyophilized	1 vial (100 ng)	Synthetic rat PYY
3. Labeled Antigen	Lyophilized	1 bottle (1.5 ng)	Biotinylated rat PYY
4. PYY Antibody	Lyophilized	1 vial	Rabbit anti-rat PYY antibody
5. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-Labeled Streptoavidin
6. Substrate Buffer	Liquid	1 bottle (25 mL)	Citrate buffer with 0.015% Hydrogen peroxide
7. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
8. Stop Solution	Liquid	1 bottle (12 mL)	1 M H_2SO_4
9. Buffer Solution Concentrate	Liquid	1 bottle (12 mL)	Phosphate buffer
10. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
11. Plate Seal		3 sheets	

MTP[*1]..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 nm
- Rotator for microtiter plate
- Washing device for microtiter plate and dispenser with an aspiration system.
- Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
- 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 a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each sample and calibrator to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Buffer Solution: Add 10 mL of the Buffer Solution Concentrate to 30 mL distilled water. This makes 40 mL of diluted Buffer Solution.

2. Preparation of Calibrator Solutions: Reconstitute the PYY Calibrator (lyophilized rat PYY, 100 ng/vial) with 1 mL of diluted Buffer Solution, giving a 100 ng/mL Calibrator Solution after reconstitution. 0.1 mL of the reconstituted Calibrator Solution is diluted with 0.2 mL of diluted Buffer Solution to yield a 33.33 ng/mL Calibrator Solution. Then, 0.1 mL of the 33.33 ng/mL Calibrator Solution is diluted with 0.2 mL of diluted Buffer Solution to yield a 11.11 ng/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 3.70, 1.23, 0.41, 0.14 ng/mL. Diluted Buffer Solution is used as the zero calibrator (0 ng/mL).

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.

3. Preparation of Labeled Antigen: Reconstitute the Labeled Antigen with 6 mL of distilled water.

   Note: Labeled Antigen must be prepared immediately before assay. Use clean test tubes or vessels.

4. Preparation of PYY Antibody: Reconstitute the PYY Antibody with 12 mL of distilled water.

   Note: PYY Antibody must be prepared immediately before assay. Use clean test tubes or vessels.

5. Preparation of Substrate Solution: Dissolve one OPD Tablet in 12 mL of Substrate Buffer.

   Note: Substrate Solution must be prepared immediately before assay. Use clean test tubes or vessels.

6. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water. Diluted Wash Solution is stable for 6 months at 4°C.

   Note: During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted.

7. Other reagents are ready for use.

STORAGE

Store kit at 4°C. Reconstituted calibrator, labeled antigen and PYY antibody should be stored at or below -30°C if dividing usage of kit.

SPECIMEN COLLECTION AND HANDLING

Plasma samples must be used as soon as possible after collection. Add 1 mg EDTA to 1 mL blood sample. If the samples are to be tested at a later time, they should be divided into test tubes in small amounts and frozen at or below -30℃. Avoid repeated freeze/thaw cycles. 100 µL is sufficient amount for the determination.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20-30°C) before beginning the test.
2. Add 350 µL/well of diluted Wash Solution into the wells and aspirate the Washing Solution. Repeat this washing procedure twice, for a total of 3 washing steps.
3. Add 50 µL of diluted Buffer Solution into wells. Then add 25 µL of the prepared Calibrator Solutions (0, 0.14, 0.41, 1.23, 3.70, 11.11, 33.33, 100 ng/mL) or samples. Next, add 50 µL of Labeled Antigen, then add 100 µL of PYY Antibody into the wells.
4. Cover the plate with a Plate Seal and incubate at room temperature for 16-20 hours. During incubation, the plate should be rotated on a plate rotator.
5. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells three times with approximately 0.35 mL/well of diluted Wash Solution.
6. Pipette 100 µL of SA-HRP Solution into each of the wells.
7. Cover the plate with a Plate Seal and incubate at room temperature for 2 hours. During the incubation, the plate should be rotated on a plate rotator.
8. Remove the Plate Seal, aspirate and wash the wells four times with approximately 0.35 mL/well of diluted Wash Solution.
9. Add 100 µL of Substrate Solution (dissolved OPD tablet) into the wells, cover the plate with a Plate Seal and incubate for 30 minutes at room temperature.
10. Add 100 µL of Stop Solution into the wells to stop the color reaction.
11. Read the optical absorbance of the wells at 490 nm. The optical absorbance of reaction solution in wells should be read as soon as possible after stopping the color reaction.

Note: Perform all determinations in duplicate.

During incubation except color reaction, the microtiter plate should be shaken gently by a microtiter plate shaker to promote immunoreaction.

RESULTS

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

When a sample value exceeds 100 ng/mL, it must be diluted with diluted Buffer Solution and re-assayed until the sample value is within the assay range.

PERFORMANCE

Analytical Recovery

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

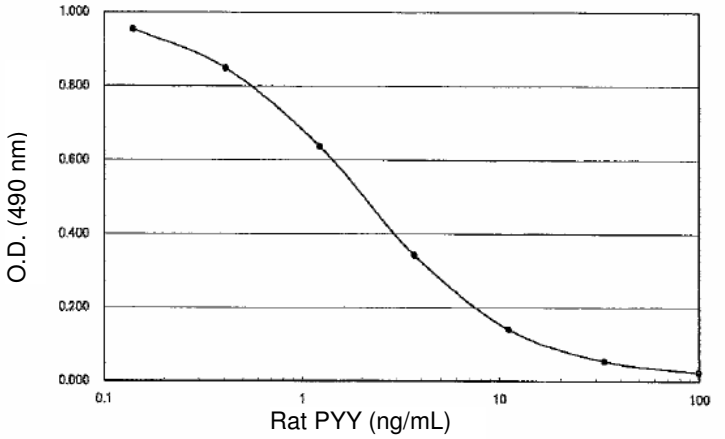
Assay Range

0.14 – 100 ng/mL

Cross-Reactivity

PYY, human: 10%
NPY, human: < 0.01%
NPY, rat: < 0.01%

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



FOR RESEARCH USE ONLY

KAMIYA BIOMEDICAL COMPANY
12779 Gateway Drive, Seattle, WA 98168
Tel: (206) 575-8068 Fax: (206) 575-8094
Email: LifeScience@k-assay.com
www.k-assay.com