Version 22-04/24

User's Manual

Rat PYY ELISA Kit

REF **DEIA4773**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This ELISA kit is used for quantitative determination of mouse/rat PYY (1-36) and mouse/rat PYY (3-36) in mouse/rat plasma or serum samples.

General Description

This ELISA 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 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) showing 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 possibly are due to the decrease in number of the endocrine cells secreting PYY. The EIA kit is prepared by using synthetic mouse/rat PYY (3-36) as standard antigen and biotinylated mouse/rat PYY (3-36) as labeled antigen. The kit contains specific polyclonal antibody recognized to the amino acid sequence of Mouse/rat PYY. The mouse/rat PYY sequence:

Y-P-A-K-P-E-A-P-G-E-D-A-S-P-E-E-L-S-R-Y-Y-A-S-L-R-H-Y-L-N-L-V-T-R-Q-R-Y-NH2

Principles of Testing

This ELISA kit for determination of PYY in mouse/rat plasma or serum samples is based on a competitive enzyme immunoassay using the combination of antibody to mouse/rat PYY and biotin–avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG, to which biotin labeled antigen, standard antigen or samples and rabbit anti mouse/rat PYY antibody are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptavidin (SA) is added to form HRP labeled SA-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 mouse/rat PYY is calculated.

Reagents And Materials Provided

1. Antibody coated plate, Microtiter plate, 1 Plate (96 wells). Main Ingredient: Goat anti rabbit IgG
2. Standard, Lyophilized powder, 1 Vial (12.5 ng). Main Ingredient: Synthetic mouse/rat PYY (3-36)
3. Labeled antigen, Lyophilized powder, 1 Vial. Main Ingredient: Biotinylated mouse/rat PYY (3-36)
4. Specific antibody, Liquid, 1 Bottle (8.5 mL). Main Ingredient: Rabbit anti mouse/rat PYY antibody
5. SA-HRP solution, Liquid, 1 Bottle (12 mL). Main Ingredient: HRP labeled streptavidin
6. Enzyme substrate solution (TMB) Liquid, 1 Bottle (12 mL). Main Ingredient: 3,3',5,5'-Tetramethylbenzidine (TMB)
7. Stopping solution, Liquid 1, Bottle (12 mL). Main Ingredient: 1M H_2SO_4
8. Buffer solution, Liquid, 1 Bottle (25 mL). Main Ingredient: BSA containing saline buffer
9. Washing solution (concentrated), Liquid, 1 Bottle (50 mL). Main Ingredient: Concentrated saline

10. Adhesive foil, 3 Sheets

Materials Required But Not Supplied

1. Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 450 nm
2. Washing device for microtiter plate and dispenser with aspiration system, but not essential
3. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips
4. Microtiter plate shaker
5. Glass test tubes for preparation of standard solution
6. Graduated cylinder (1000 mL)
7. Distilled 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 (12.5 ng/vial) with 1 mL of buffer solution, which affords 12.5 ng/mL standard solution. The reconstituted standard solution (0.1 mL) is diluted with 0.2 mL of buffer solution that yields 4.17 ng/mL standard solution. Repeat the same dilution to make each standard solution of 1.39, 0.46, and 0.15 ng/mL. Buffer solution is used as 0 ng/mL (Bo).

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

Reconstitute Labeled antigen with 7 mL of Buffer solution.

3. **Preparation of washing solution:**

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

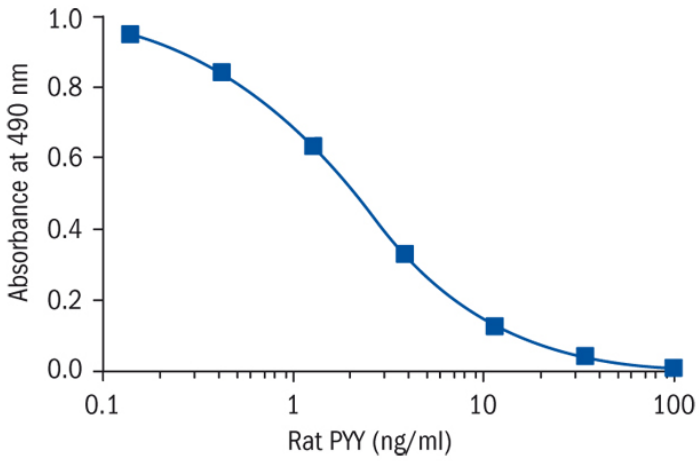
4. **Other reagents are ready for use.**

Assay Procedure

1. Before starting assay, bring all the reagents except samples to room temperature (20-30 °C).
2. Introduce 350 µL of washing solution to each well and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times). Finally, invert the plate and firmly tap it onto an absorbent surface, such as paper toweling, to endure blotting free of most residual washing solution.
3. Pipette 50 µL of labeled antigen solution into each well first, then introduce 25 µL of each of standard solutions (0, 0.15, 0.46, 1.39, 4.17, 12.5 ng/mL) or samples and finally add 75 µL of specific antibody solution into the wells.
4. Cover the plate with adhesive foil and incubate it at 4°C for 18 hours(±1 hour) without shaking and further more 30 minutes at room temperature with shaking (100-150 rpm).

5. After incubation, take off the adhesive foil, aspirate and wash the well 5 times with 350 µL of washing solution. Finally, invert the plate and firmly tap it onto an absorbent surface, such as paper toweling, to endure blotting free of most residual washing solution.
6. Add 100 µL of SA-HRP solution into each of the wells.
7. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour with shaking (100-150 rpm).
8. Take off the adhesive foil, aspirate and wash the well 5 times with 350 µL of washing solution. Finally, invert the plate and firmly tap it onto an absorbent surface, such as paper toweling, to endure blotting free of most residual washing solution.
9. Add 100 µL of Enzyme substrate solution (TMB) into each well; cover the plate with adhesive foil and keep it for 30 minutes at room temperature in a dark place for color reaction without shaking.
10. Add 100 µL of stopping solution into each well to stop color reaction.
11. Read the optical absorbance of the wells at 450 nm immediately.
12. Calculate mean absorbance values of standards and plot a standard curve on semi-logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to read mouse/rat PYY concentrations in samples from the corresponding absorbance values. If an immunoassay software package be used, we recommend that the data be handled by utilizing a 4-parameter logistic curve fitting program.

Typical Standard Curve



Detection Range

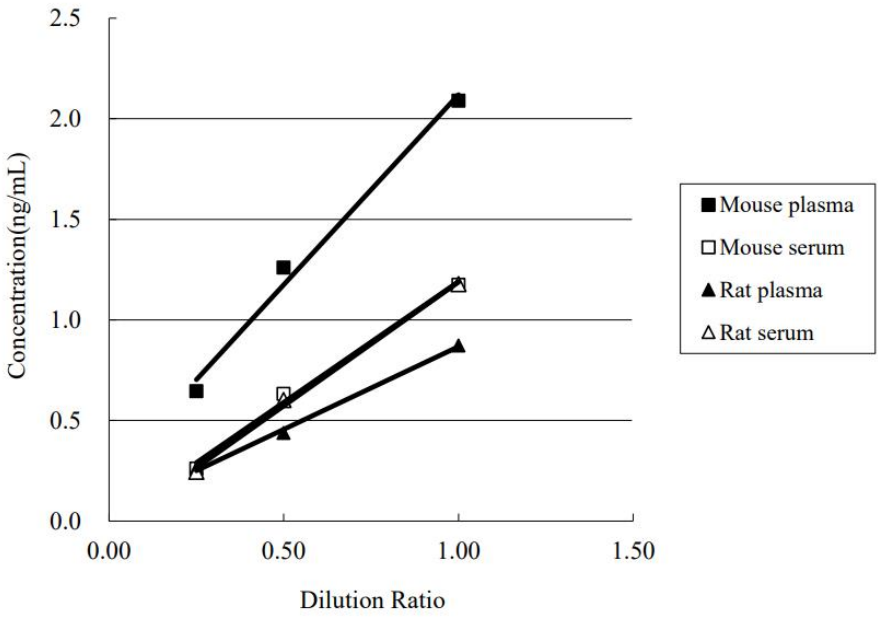
0.15-12,5 ng/ml

Specificity

The ELISA kit shows 100% cross reactivity to mouse/rat PYY (3-36) and 115% to mouse/rat PYY (1-36). Cross reactivity was not observed in the assay range with mouse/rat NPY that has similar amino acid

sequence with mouse/rat PYY. No cross reactivity with GLP-1 (7-36)-NH2, GLP-1 (1-37), and rat GLP-2 were observed.

Linearity



Satisfactory dilution characteristics were shown with mouse and rat samples.

Recovery

1. Rat serum 1

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	0.965		
0.5	1.447	1.465	98.7
2.0	2.748	2.965	92.7
5.0	5.077	5.965	85.1

2. Rat serum 2

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	1.107		
0.5	1,510	1.607	94.0
2.0	3.274	3.107	105.4
5.0	5.395	6.107	88.3

3. Rat plasma 1

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	0.718		
0.5	1.111	1.218	91.2
2.0	2.407	2.718	85.9
5.0	5.059	5.718	81.8

4. Rat plasma 2

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	0.595		
0.5	0.898	1.095	82.0
2.0	2.543	2.595	98.0
5.0	4.605	5.595	82.3

5. Mouse serum 1

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	1.987		
0.5	2.633	2.487	105.9
2.0	5.052	3.987	126.7
5.0	9.009	6.987	128.9

6. Mouse serum 2

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	1.867		
0.5	2.562	2.367	108.2
2.0	5.029	3.867	130.1
5.0	9.547	6.867	139.0

7. Mouse plasma 1

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	0.766		
0.5	1.153	1.266	91.1
2.0	2.645	2.766	95.6
5.0	5.647	5.766	97.9

8. Mouse plasma 2

Added PYY (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	1.466		
0.5	1.926	1.966	97.9
2.0	4.050	3.466	116.8
5.0	7.721	6.466	119.4

Reproducibility

Intra-assay CV (%): 3.1-9.8

Inter-assay CV (%): 4.2-14.2

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 blood, yielding 100 μM final concentration. EDTA-2Na additive blood collection tube is recommended for the plasma collection. It is recommended that serum or plasma samples should be tested as soon as possible after separation. If the sample is tested later, they should be aliquoted and frozen below –30°C (for long term storage, it is recommended that the sample should be stored in a -70°C deep freezer). Avoid repeated freezing and thawing of samples. Samples should be kept in an ice bath after thawing before the assay and used within 60 minutes.
2. Standard and labeled antigen solutions should be prepared immediately before use. The plate can be used for separately twice. In that case, the reconstituted reagents (standard and labeled antigen solution) should be stored at or below -30°C.
3. During storage of washing solution (concentrated) at 2-8°C, precipitates may be observed occasionally, however they will be dissolved when diluted.
4. As pipetting operations may affect accuracy and precision of the assay, pipette solutions especially for standard solutions or samples precisely into each well of the plate. In addition, use clean test tubes or vessels in assay and use a new tip for each sample or standard solution and for each step of preparation of the standard diluting solution to avoid cross contamination.
5. Perform all the determination in duplicate.
6. If an over range (over than 12.5 ng/mL) sample be tested or predicted, dilute this sample with assay buffer, then re-operate the assay procedure.
7. To quantitate accurately, always run a standard curve when measuring samples.
8. Color reaction should be carried out under the light proof condition.
9. Read optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction. Shake the plate for 5-10 seconds to mix the contents before reading the absorbance.
10. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
11. Satisfactory performance of the assay will be guaranteed only when reagents are used from combination pack with identical lot number.