



PYY (Mouse, Rat) ELISA

For the quantitative determination of peptide YY (PYY) in mouse/rat plasma or serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number:	48-PYYRT-E01.1
Size:	96 wells
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I. Introduction

This enzyme linked immunosorbent assay (ELISA) is a stable and convenient assay system for peptide YY (PYY). PYY was isolated initially by Tatemoto et al. (1980) from 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 juices. PYY is released by food intake. The PYY level decreases after resection of the intestine, possibly due to the decrease in number of the endocrine cells secreting PYY.

This ELISA kit is prepared by using synthetic mouse/rat PYY (3-36) as the standard antigen and biotinylated mouse/rat PYY (3-36) as the labeled antigen. The kit contains specific polyclonal antibodies that recognize the amino acid sequence of both the mouse/rat PYY (1-36) and (3-36) fragments.

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

PYY (Rat) ELISA	Contents
<ul style="list-style-type: none">The assay measures mouse/rat PYY in the range of 0.15-12.5 ng/mL.	1) Antibody coated plate
<ul style="list-style-type: none">The assay can be completed within 18.5 hr + 1.5 hr.	2) Standard
<ul style="list-style-type: none">With one assay kit, 42 samples can be measured in duplicate.	3) Labeled antigen
<ul style="list-style-type: none">Test sample: mouse/rat plasma or serum	4) Specific antibody
Sampe volume: 25 µl	5) SA-HRP solution
<ul style="list-style-type: none">The 96-well plate consists of 12 8-well strips. The strips can be separated.	6) Enzyme substrate solution (TMB)
	7) Stop Solution
<ul style="list-style-type: none">Intra-assay CV (%) 3.1-9.8	8) Buffer solution
Inter-assay CV (%) 4.2-14.2	9) Wash solution (concentrated)
	10) Adhesive foil
<ul style="list-style-type: none">Store all of the components at 2-8°C.	
<ul style="list-style-type: none">The kits are stable for 9 months from the date of manufacturing.	
The expiration date is on the box label.	

II. Characteristics

This ELISA kit is used for the quantitative determination of mouse/rat PYY (1-36) and mouse/rat PYY (3-36) in mouse/rat plasma or serum samples. There are many advantages to performing this assay including sensitive quantification, high specificity, no influence from other sample components, and the needlessness of sample pretreatment. The standard is a highly purified synthetic product (purity: higher than 98%).

Specificity

This 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, which has a similar amino acid sequence to mouse/rat PYY. No cross-reactivity with GLP-1 (7-36)-NH₂, GLP-1 (1-37), and rat GLP-2 was observed.

Assay Principle

This ELISA for the determination of PYY in mouse/rat plasma or serum samples is based on a competitive enzyme immunosorbent assay that uses a combination of an antibody to mouse/rat PYY and a biotin-avidin affinity system. The 96-well plate is coated with goat anti-rabbit IgG. Standards or samples, biotin labeled antigen, and rabbit anti-mouse/rat PYY antibody are added to the wells for a competitive immunoreaction. After incubation and plate washing, horseradish peroxidase (HRP) labeled streptavidin (SA) is added to form HRP labeled SA-biotinylated antigen-antibody complexes 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.

III. Composition

<i>Component</i>	<i>Form</i>	<i>Quantity</i>	<i>Main Ingredient</i>
1. Antibody coated plate	Microtiter plate	1 plate (96 wells)	Goat anti-rabbit IgG
2. Standard	Lyophilized powder	1 vial (12.5 ng)	Synthetic mouse/rat PYY (3-36)
3. Labeled antigen	Lyophilized powder	1 vial	Biotinylated mouse/rat PYY (3-36)
4. Specific antibody	liquid	1 bottle (8.5 mL)	Rabbit anti-mouse/rat PYY antibody
5. SA-HRP solution	liquid	1 bottle (12 mL)	HRP labeled SA
6. Enzyme substrate solution	liquid	1 bottle (12 mL)	3,3',5,5'-tetramethylbenzidine (TMB)
7. Stop solution	liquid	1 bottle (12 mL)	1 M H ₂ SO ₄
8. Buffer solution	liquid	1 bottle (25 mL)	BSA containing saline buffer
9. Wash solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
10. Adhesive foils		3 sheets	

IV. Method

Equipment required but not supplied

- 1) Photometer for microtiter plate (plate reader), which can read extinction 2.5 at 490 nm
- 2) Microtiter plate shaker
- 3) Washing device for microtiter plate and dispenser with aspiration system (recommended)
- 4) Micropipettes, multi-channel pipettes for 8 wells or 12 wells and appropriate tips

- 5) Glass test tubes for preparation of standard solution
- 6) Graduated cylinder (1,000 mL)
- 7) Distilled water or deionized water
- 8) DPP IV inhibitor (recommended when measuring only PYY (3-36), see below)
- 9) EDTA-2Na additive blood collection tubes (recommended for plasma collection, see below)

Preparatory work

1) Samples

If only PYY (3-36) is being measured in blood sample, DPP IV inhibitor should be added immediately to the blood, to yield a 100 μM final concentration. EDTA-2Na additive blood collection tubes are recommended for the collection of plasma. Serum or plasma samples should be tested as soon as possible after separation. If the sample is to be tested later, the samples should be stored frozen below -30°C in aliquots (for longer term storage, it is recommended that the samples be stored in a deep freezer at -70°C). Avoid repeated freezing and thawing of the samples. Samples should be kept in an ice bath after thawing before the assay and used within 60 minutes.

2) Standard solutions

Reconstitute the standard (12.5 ng/vial) with 1 mL of Buffer solution, which yields the 12.5 ng/mL standard solution. Dilute 0.1 mL of the 12.5 ng/mL standard solution with 0.2 mL of buffer solution to yield the 4.17 ng/mL standard solution. Repeat the dilution to make each standard solution: 1.39, 0.46, and 0.15 ng/mL. Buffer solution is used as the 0 ng/mL standard solution (B_0).

3) Labeled antigen solution

Reconstitute labeled antigen with 7 mL of buffer solution.

4) Wash solution:

Dilute 50 mL of Wash solution (concentrated) to 1,000 mL with distilled or deionized water.

5) Other reagents are ready for use.

Procedure

1. Bring all of the reagents, except the samples, to room temperature ($20\text{-}30^{\circ}\text{C}$) before beginning the assay.
2. Add 350 μl of wash solution (diluted) to each well, and then aspirate the wash solution in the wells. Repeat this procedure twice for a total of three washes. Finally, invert the plate and firmly tap it onto an absorbent surface, such as paper towels, to blot away most of the residual wash solution.
3. Pipette 50 μl of labeled antigen solution into the wells first, then add 25 μl each of the standard solutions (0, 0.15, 0.46, 1.39, 4.17, 12.5 ng/mL) or samples. Finally add 75 μl of specific antibody to the wells.
4. Cover the plate with adhesive foil and incubate at 4°C for 18 hours (\pm 1 hour) without shaking. Next, incubate for 30 minutes at room temperature with shaking (100-150 rpm).
5. After incubation, take off the adhesive foil, aspirate the solution in the wells, and wash the wells five times with 350 μl of wash solution (diluted). Invert the plate and firmly tap it onto an absorbent surface, such as paper towels, to blot away most of the residual wash solution.

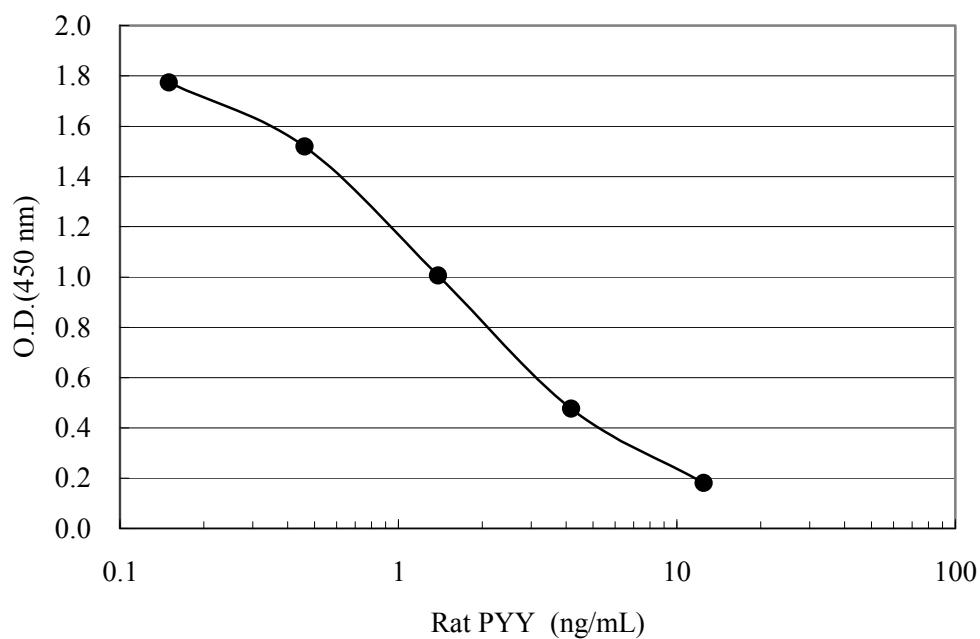
6. Pipette 100 μ l of SA-HRP solution to each of the wells.
7. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour with shaking (100-150 rpm).
8. Take off the adhesive foil, aspirate the solution in the wells, and wash the wells five times with 350 μ l of wash solution (diluted). Invert the plate and firmly tap it onto an absorbent surface, such as paper towels, to blot away most of the residual wash solution.
9. Add 100 μ l of enzyme substrate solution (TMB) to the wells. Cover the plate with adhesive foil and incubate for 30 minutes at room temperature, protected from light. (The plate should not be shaken.)
10. Add 100 μ l of Stop solution to the wells.
11. Read the optical absorbance of the wells at 450 nm immediately. (Shake the plate for 5-10 seconds to mix the contents before reading the absorbance.)
12. Calculate the mean absorbance values of the standards and plot a standard curve on semi-logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to determine the mouse/rat PYY concentrations of the samples from the corresponding absorbance values. If an immunoassay software package is used, it is recommended that the data be handled by utilizing a 4-parameter logistic curve fit.

V. Notes

- 1) Standard and labeled antigen solutions should be prepared immediately before use. The microplate strips can be divided and used separately. The reconstituted reagents (standards, labeled antigen) should be stored at or below -30°C .
- 2) During storage of Wash solution (concentrated) at $2-8^{\circ}\text{C}$, precipitates may be observed; however, they will be dissolved when diluted.
- 3) Pipetting operations may affect the precision of the assay. Pipette standard solutions and samples precisely into each well of the plate. Use clean test tubes or vessels and use a new tip for each sample to avoid cross contamination.
- 4) Perform all the determinations in duplicate.
- 5) When a sample value exceeds 12.5 ng/mL, dilute the sample with Buffer solution to a concentration within the range and assay again.
- 6) To quantify accurately, always run a standard curve when testing samples.
- 7) During the color reaction incubation (step 9) the plate should be protected from light.
- 8) Read the optical absorbance of the wells as soon as possible after stopping (step 10) the color reaction. Shake the plate for 5-10 seconds to mix the contents before reading the absorbance.
- 9) Protect the reagents from strong light (e.g., direct sunlight) during storage and assay.
- 10) Satisfactory performance of the test is guaranteed only when reagents are used with identical lot numbers.

VI. Performance Characteristics

<Typical standard curve>



< Precision and reproducibility >

Intra-assay CV (%): 3.1 ~ 9.8

Inter-assay CV (%): 4.2 ~ 14.2

< Analytical recovery >

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

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

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

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

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

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

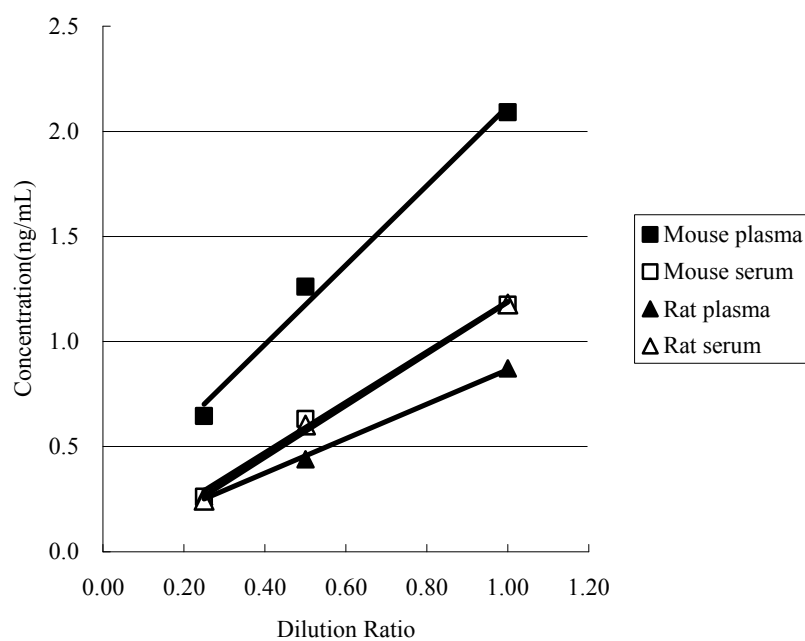
Mouse plasma 1

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

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

<Dilution test>



Satisfactory dilution characteristics were shown with mouse and rat samples.

References

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