COSMO BIO CO., LTD.
Inspiration for Life Science

Rat PYY EIA Kit

Cat.No. YII-YK081-EX

FOR LABORATORY USE ONLY

Distributor
COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618

Contents

- Please read all the package insert carefully before beginning the assay -

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 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 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 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.

YK081 Rat PYY EIA Kit

- ▼ The assay kit can measure rat PYY in the range of 0.14-100 ng/mL
- ▼ The assay completes within 16-20 hr.＋2.5 hr.
- ▼ With one assay kit, 40 samples can be measured in duplicate
- ▼ Test sample: rat plasma
  Sample volume: 25 μL
- ▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV(%) 7.95 - 12.81
  Inter-assay CV(%) 11.95 - 13.61
- ▼ Stability and Storage
  Store all of the components at 2-8℃.
  6 months from the date of manufacturing.
  The expiry date is described on the label of kit.

Contents

1) Antibody coated plate
2) Rat PYY standard
3) Labeled antigen
4) PYY antibody
5) SA-HRP solution
6) Substrate buffer
7) OPD tablet
8) Stopping solution
9) Buffer solution (concentrated)
10) Washing solution (concentrated)
11) Adhesive foil

II. Characteristics

This EIA kit is used for quantitative determination of rat PYY in rat plasma sample. The kit is characterized for sensitive quantification, high specificity and no influence with other components in plasma and needlessness of sample pre-treatment. PYY standard used in the kit is highly purified synthetic product (purity: higher than 98%).

< Specificity>

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

< Test Principle>

This EIA kit for determination of rat PYY in plasma sample is based on the competitive enzyme immunoassay using combination of highly specific antibody to rat PYY and biotin-avidin affinity system. The 96 wells plate is coated with goat anti rabbit IgG. PYY standard or samples, labeled antigen and anti rat PYY antibody are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptoavidin are added to form HRP labeled streptoavidin-biotinylated rat PYY-antibody complex on the surface on the wells. Finally, HRP enzyme activity is determined by o-phenylenediamine dihydrochloride (OPD) 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)	Goat Anti rabbit IgG
2. Rat PYY standard	lyophilized	1 vial (100ng)	Synthetic rat PYY
3. Labeled antigen	lyophilized	1 vial (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 containing 0.015% hydrogen peroxide
7. OPD tablet	tablet	2 tablets	o-Phenylenediamine dihydrochloride
8. Stopping solution	liquid	1 bottle (12 mL)	1M 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]……Microtiter plate

Ⅳ. Method

< Equipment required>

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
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), Assay it as fresh as possible. 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. 100 μL is sufficient amount for the determination.

2. Preparation of buffer solution:
   10 mL of buffer solution (concentrated) is to be diluted with 30mL of distilled water, which makes 40 mL 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. The 0.1ml of the reconstituted standard solution is diluted with 0.2 mL of diluted buffer solution that yields 33.33 ng/mL standard solution. The 0.1mL of 33.33 ng/mL standard solution is diluted with 0.2 mL of the diluted buffer solution, which makes 11.11 ng/mL standard solution. Repeat the dilution to make each standard of 3.70, 1.23, 0.41, 0.14 ng/mL. Diluted buffer solution is used as 0 ng/mL.

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

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

6. Preparation of substrate solution:
   Resolve one 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. Bring all the reagents and samples to room temperature (20-30°C) 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). 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 50μL of buffer solution into wells first, then introduce 25μL each of standard solutions (0, 0.14, 0.41, 1.23, 3.70, 11.11, 33.33, 100 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 shaken with a microtiter plate shaker.

5. Take off the adhesive foil, aspirate the solution in the wells and wash the wells 3 times with approximately 0.35 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 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 shaken with a plate shaker.

8. Take off the adhesive foil, aspirate and wash the wells 4 times with approximately 0.35mL/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 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 color reaction.

11. Read optical absorbance of the solution in the wells at 490 nm. Calculate mean absorbance values of standard solution and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance value). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

V. Notes

1. 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°C. Avoid repeated freezing and thawing of plasma samples.

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

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

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

5. When sample value exceeds 30 ng/mL, it needs to be diluted with buffer solution to proper concentration.

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

7. Perform all the determination in duplicate.

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

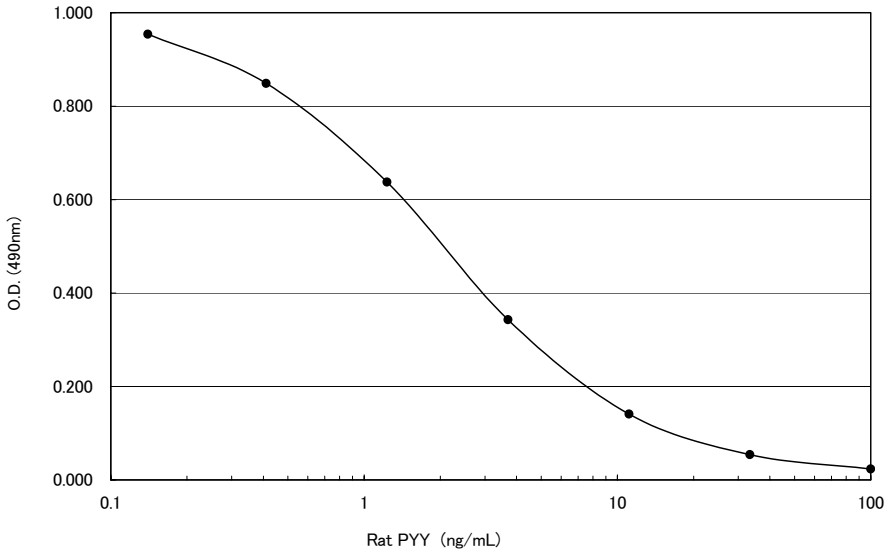
9. To quantitate accurately, always run a standard curve when testing samples.

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

11. 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

0.14 − 100 ng / mL

VII. Stability and Storage

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

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

< Package > For 96 tests per one kit including standards

VIII. References

1. Adrin, T.E., Smith, H.A, Calvert, S.A., Aynsley-Green, A.and Bloom, S.R. (1986): Elevated plasma peptide YY in human neonates and infants. *Pediatric Res.,**20:*** 1225 -1227.

2. Adrian T.E., Ferri, G.L., Bacarese-Hamilton, A.J., Fuessl, H.S., Polak, J.M. and Bloom, S.R. (1995): Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology,**89*:** 1070-1077.

3. El-Salhy, M., Grimelius, L ., Wilander, E ., Ryberg B., Terenius, L., Lundburg, J.M.and Tatemoto, K. (1983): Immunocytochemical identification of polypeptide YY(PYY) cells in the human gastrointestinal tract. *Histochemistry, 77*:15-23.

4. Greeley, G.H.Jr., Hashimoto, T., Izukawa, M., Gomez, G., Jeng, J. Hill, F.L.C., Liuis, F.,and Tompson, J/C. (1989): A comparison of intraduodenally and intracolonically administered nutrients on the release of peptide YY in the dog. *Endrocrinology, **125***:1761-1765.

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.

6. Gomez, G., Zhang, T., Rajaraman, S., Thakore, K.N., Yanaihara, N., Townsendand C.M.Jr., Tompson, J.C. and Greeley, G.H.Jr. (1995): Intestinal peptide YY : ontogeny of gene expression in rat bowel and trophic action on rat and mouse bowel. *Am J.Physiol. **268***, G71-G81.

7. Larhammar, D. (1996): Evolution of neuropeptide Y, peptide YY and pancreatic polypeptide: *Regul.Pept., **62***, 1-11.

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO EKIMAE BLDG, 2-20,TOYO 2CHOME,KOTO-KU,TOKYO 135-0016,JAPAN
TEL: +81-3-5632-9617 FAX: +81-3-5632-9618 e-mail: export@cosmobio.co.jp
www.cosmobio.co.jp