RayBio®
Human/Mouse/Rat PYY
Enzyme Immunoassay Kit

Please Read the Manual Carefully
Before Starting your Experiment

User Manual 3.2
(Revised June 11, 2013)

RayBio® PYY Enzyme
Immunoassay Kit Protocol

(Cat#: EIA-PYY-1)

RayBiotech, Inc.

We Provide You With Excellent
Protein Array System and Service

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I. INTRODUCTION

Peptide YY is a 36 amino acid peptide released by cells in the ileum and colon in response to feeding. It is also known as PYY, Peptide Tyrosine Tyrosine, or Pancreatic Peptide YY_{3-36}.

There are two major forms of Peptide YY: PYY_{1-36} and PYY_{3-36} which is the most common form of circulating PYY. Peptide YY_{3-36} (PYY) is a linear polypeptide consisting of 36 amino acids with structural homology to NPY and pancreatic polypeptide. Circulating PYY concentration increases postprandially and decreases by fasting.

PYY exerts its action through NPY receptors, inhibits gastric motility and increases water and electrolyte absorption in the colon. PYY may also suppress pancreatic secretion. It is secreted by the neuroendocrine cells in the ileum and colon in response to a meal, and has been shown to reduce appetite. PYY works by slowing the gastric emptying; hence, it increases efficiency of digestion and nutrient absorption after meal.

PYY has been shown to play an important role in obesity. Animal studies have shown that acute peripheral administration of PYY_{3-36} inhibits feeding of rodents and primates. Studies on Y2R-knockout mice have revealed that there is no anorectic effect on Y2R-knockot mice (Y2R is the receptor for PYY). These findings indicate that PYY_{3-36} has anorectic effect which is suggested to be mediated by Y2R. Studies on PYY-knockout mice have shown that they have higher fat mass and lower glucose tolerance when compared to control mice, indicating that PYY also plays very important role in energy homeostasis by balancing the food intake.

Studies have also shown that obese people secrete less PYY than non-obese people. The anorectic effect of PYY represents a possible anti-obesity therapy in the future.

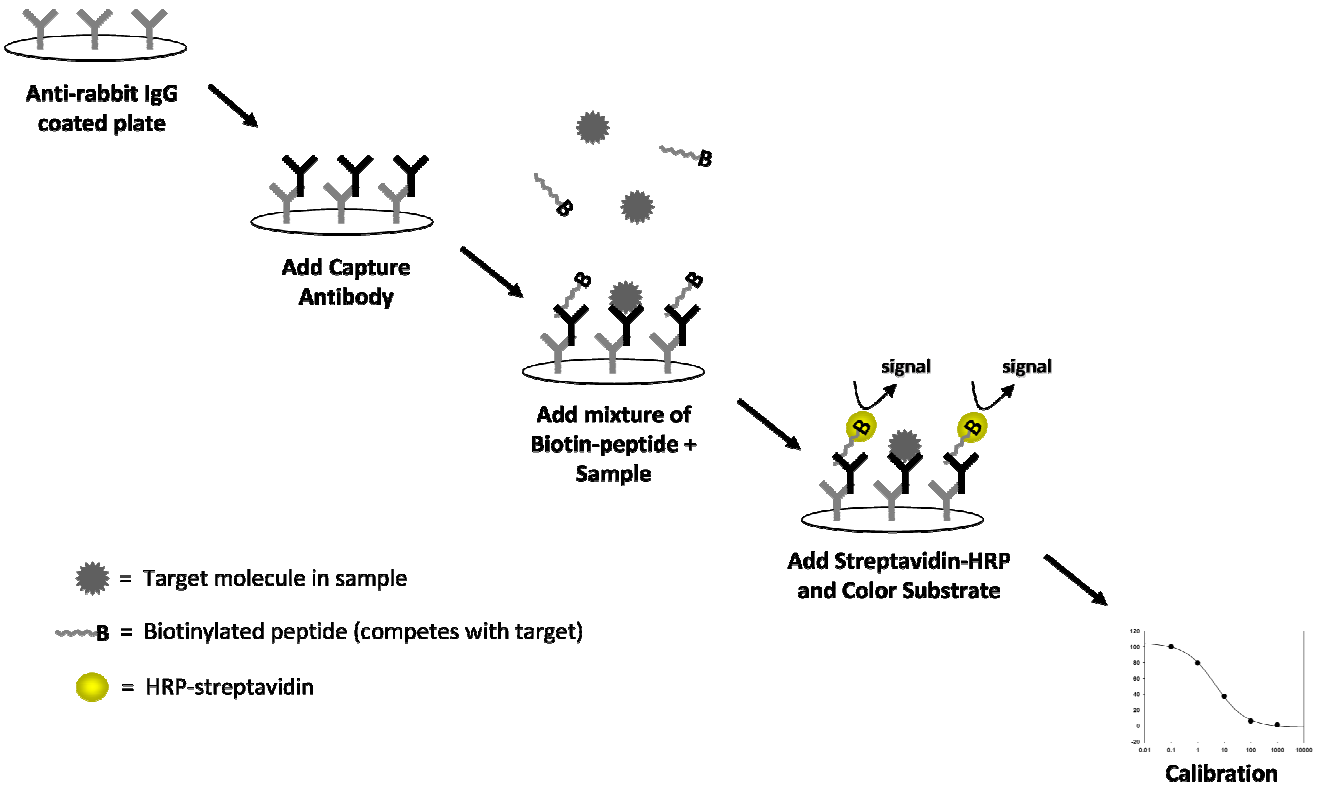
II. GENERAL DESCRIPTION

The RayBio® PYY Enzyme Immunoassay (EIA) Kit is an in vitro quantitative assay for detecting PYY peptide based on the principle of Competitive Enzyme Immunoassay.

The microplate in the kit is pre-coated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-PYY antibody, both biotinylated PYY peptide and peptide standard or targeted peptide in samples interacts competitively with the PYY antibody. Uncompeted (bound) biotinylated PYY peptide then interacts with Streptavidin-horseradish peroxidase (SA-HRP), which catalyzes a color development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of PYY peptide in the standard or samples. This is due to the competitive binding to PYY antibody between biotinylated PYY peptide and peptides in standard or samples. A standard curve of known concentration of PYY peptide can be established and the concentration of PYY peptide in the samples can be calculated accordingly.

EIA-PYY-1 detects the 1-36 form. The 3-36 form may also be detected, but this has not been conclusively tested.

Principle of Competitive EIA



III. REAGENTS

1. PYY Microplate (Item A): 96 wells (12 strips x 8 wells) coated with secondary antibody.
2. Wash Buffer Concentrate (20x) (Item B): 25 ml.
3. Lyophilized standard PYY peptide (Item C): 2 vials.
4. Lyophilized anti-PYY polyclonal antibody (Item N): 2 vials.
5. Assay Diluent A (Item D): 30 ml, contains 0.09% sodium azide as preservative. Diluent for standards and samples.
6. Assay Diluent B (Item E): 15 ml of 5x concentrated buffer. Diluent for anti-PYY antibody and HRP-Streptavidin.
7. Lyophilized biotinylated PYY peptide (Item F): 2 vials.
8. HRP-Streptavidin concentrate (Item G): 600 µl 60x concentrated HRP-conjugated Streptavidin.
9. Lyophilized positive control (Item M): 1 vial.
10. TMB One-Step Substrate Reagent (Item H): 12 ml of 3, 3', 5, 5'- tetramethylbenzidine (TMB) in buffered solution.
11. Stop Solution (Item I): 8 ml of 0.2 M sulfuric acid.
12. Assay Diagram (Item J).
13. User Manual (Item K).

IV. STORAGE

- Standard, Biotinylated PYY peptide, and Positive Control should be stored at -20℃ after arrival. **Avoid multiple freeze-thaws.**
- The remaining kit components may be stored at 4℃.
- Opened Microplate Wells and antibody (Item N) may be stored for up to 1 month at 2° to 8℃. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.
- If stored in this manner, RayBiotech warranties this kit for 6 months from the date of shipment.

V. ADDITIONAL MATERIALS REQUIRED

1. Microplate reader capable of measuring absorbance at 450nm.
2. Precision pipettes to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. SigmaPlot software (or other software which can perform four-parameter logistic regression models)
8. Tubes to prepare standard or sample dilutions.
9. Orbital shaker
10. Aluminum foil
11. Saran Wrap

VI. REAGENT PREPARATION

For samples including sera, plasma, cell culture media or other sample types, use Assay Diluent A to dilute Item F and Item C. For sample and positive control dilutions, refer to steps 6, 7, 8 and 10 of Reagent Preparation.

1. Keep kit reagents on ice during reagent preparation steps. Equilibrate plate to room temperature before opening the sealed pouch.

2. Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.

3. Briefly centrifuge the Anti-PYY Antibody vial (Item N) and reconstitute with 5 µl of ddH_2O before use. Add 50 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently.

4. The antibody concentrate should then be diluted 100-fold with 1x Assay Diluent B. This is your anti-PYY antibody working solution, which will be used in step 2 of the Assay Procedure.

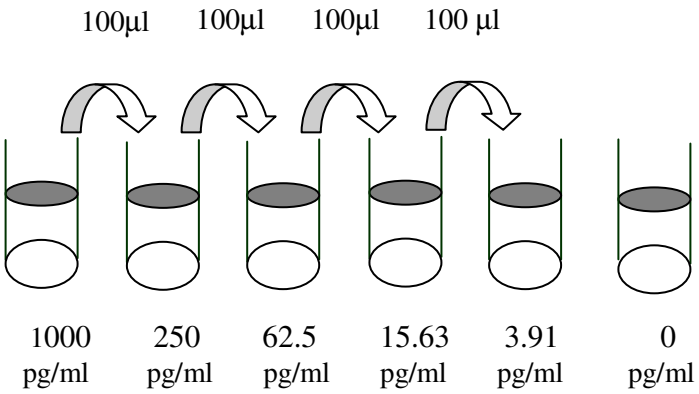
NOTE: the following steps may be done during the antibody incubation procedure (step 2 of Assay Procedure).

5. Briefly centrifuge the vial of biotinylated PYY peptide (Item F) and reconstitute with 20 µl of ddH_2O before use. Add 5 µl of Item F to 5 ml of Assay Diluent A. Pipette up and down to mix gently. *The final concentration of biotinylated PYY will be 40 pg/ml.* This solution will only be used as the diluent in step 6 of Reagent Preparation.

6. Preparation of Standards: Label 6 microtubes with the following concentrations: 1000 pg/ml, 250 pg/ml, 62.5 pg/ml, 15.6 pg/ml, 3.91 pg/ml and 0 pg/ml. Pipette 300 µl of biotinylated PYY solution into each tube, except for the 1000 pg/ml (leave this one empty). *It is very important to make sure the concentration of biotinylated PYY is 40 pg/ml in all standards.*

a. Briefly centrifuge the vial of standard PYY peptide (Item C) and reconstitute with 6 µl of ddH_2O. In the tube labeled 1000 pg/ml, pipette 6 µl of Item C and 594 µl of 40 pg/ml biotinylated PYY solution (prepared in step 5 above). This is your PYY stock solution (1000 pg/ml PYY, 40 pg/ml biotinylated PYY). Mix thoroughly. This solution serves as the first standard.
b. To make the 250 pg/ml standard, pipette 100 µl of PYY stock solution the tube labeled 250 pg/ml. Mix thoroughly.
c. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 300 µl of biotinylated PYY and 100 µl of the prior concentration until 3.91 pg/ml is reached. Mix each tube thoroughly before the next transfer.

d. The final tube (0 pg/ml PYY, 40 pg/ml biotinylated PYY) serves as the zero standard (or total binding).



7. Prepare a 10-fold dilution of Item F. To do this, add 2 µl of Item F to 18 µl of the appropriate Assay Diluent. This solution will be used in steps 8 and 10.

8. Positive Control Preparation: Briefly centrifuge the positive control vial and reconstitute with 100 µl of ddH_2O before use (Item M). To the tube of Item M, add 101 µl 1x Assay Diluent B. Also add 2 µl of 10-fold diluted Item F (prepared in step 7) to the tube. This is a 2-fold dilution of the positive control. Mix thoroughly. The positive control is a cell culture medium sample with an expected signal between 10% and 30% of total binding (70-90% competition) if diluted as described above. It may be diluted further if desired, but be sure the final concentration of biotinylated PYY is 40 pg/ml.

9. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1X Wash Buffer.

10. Sample Preparation: Use Assay Diluent A + biotinylated PYY to dilute serum/plasma samples. For cell culture medium and other sample types, use 1X Assay Diluent B + biotinylated PYY as the diluent. *It is very important to make sure the final concentration of the biotinylated PYY is 40 pg/ml in every sample.* EXAMPLE: to make a 4-fold dilution of sample, mix together 2.5 µl of 10-fold diluted Item F (prepared in step 7), 185 µl of appropriate Assay Diluent, and 62.5 µl of your sample; mix gently. The total volume is 250 µl, enough for duplicate wells on the microplate.
    Do not use Item F diluent from Step 5 for sample preparation.
    If you plan to use undiluted samples, you must still add biotinylated PYY to a final concentration of 40 pg/ml. EXAMPLE: Add 2.5 µl of 10-fold diluted Item F to 247.5 µl of sample. NOTE: Optimal sample dilution factors should be determined empirically, however you may contact technical support (888-494-8555; techsupport@raybiotech.com) to obtain recommended dilution ranges for serum or plasma.

11. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use. The HRP-Streptavidin concentrate should be diluted 60-fold with 1X Assay Diluent B.

    Note: Do not use Assay Diluent A for HRP-Streptavidin preparation in Step 11.

VII. ASSAY PROCEDURE:

1. Keep kit reagents on ice during reagent preparation steps. It is recommended that all standards and samples be run at least in duplicate.

2. Add 100 µl anti-PYY antibody (see Reagent Preparation step 4) to each well. Incubate for 1.5 hours at room temperature

with gentle shaking (1-2 cycles/sec). You may also incubate overnight at 4 degrees C.

3. Discard the solution and wash wells 4 times with 1x Wash Buffer (200-300 µl each), Washing may be done with a multichannel pipette or an automated plate washer. Complete removal of liquid at each step is essential to good assay performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 µl of each standard (see Reagent Preparation step 6), positive control (see Reagent Preparation step 8) and sample (see Reagent Preparation step 10) into appropriate wells. Be sure to include a blank well (Assay Diluent only). Cover wells and incubate for 2.5 hours at room temperature with gentle shaking (1-2 cycles/sec) or overnight at 4℃.

5. Discard the solution and wash 4 times as directed in Step 3.

6. Add 100 µl of prepared HRP-Streptavidin solution (see Reagent Preparation step 11) to each well. Incubate with gentle shaking for 45 minutes at room temperature or overnight at 4℃. It is recommended that incubation time should not be shorter or longer than 45 minutes.

7. Discard the solution and wash 4 times as directed in Step 3.
8. Add 100 µl of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking (1-2 cycles/sec).

9. Add 50 µl of Stop Solution (Item I) to each well. Read absorbances at 450 nm immediately.

VIII. ASSAY PROCEDURE SUMMARY

1. Prepare all reagents, samples and standards as instructed.

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2. Add 100 µl anti-PYY antibody to each well. Incubate 1.5 hours at room temperature or overnight at 4°C.

⇩

3. Add 100 µl standard or sample to each well. Incubate 2.5 hours at room temperature or overnight at 4°C.

⇩

4. Add 100 µl prepared streptavidin solution. Incubate 45 minutes at room temperature.

⇩

5. Add 100 µl TMB One-Step Substrate Reagent to each well. Incubate 30 minutes at room temperature.

⇩

6. Add 50 µl Stop Solution to each well. Read at 450 nm immediately

IX. CALCULATION OF RESULTS

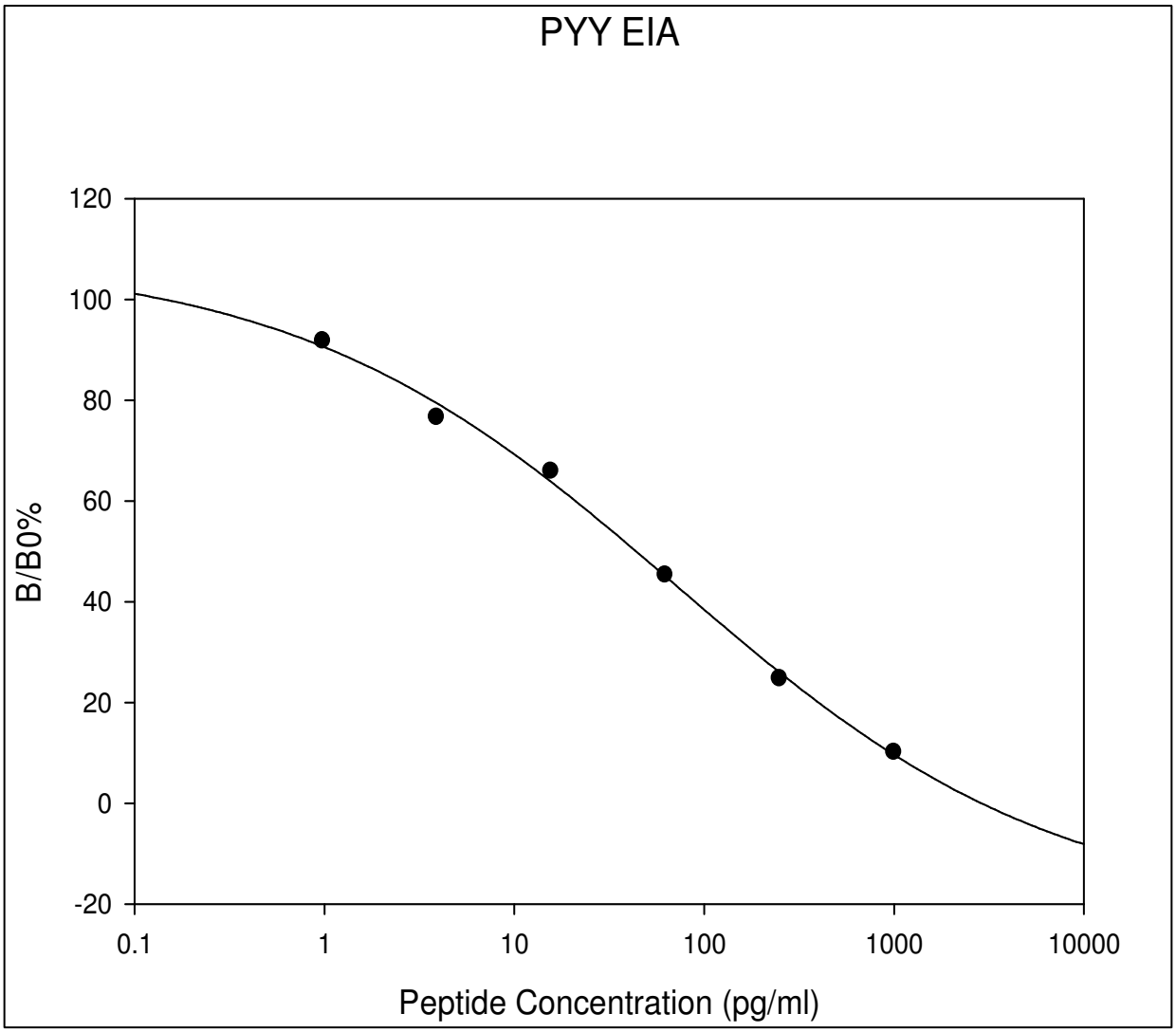
Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the blank optical density. Plot the standard curve using SigmaPlot software (or other software which can perform four-parameter logistic regression models), with standard concentration on the x-axis and percentage of absorbance (see

calculation below) on the y-axis. Draw the best-fit curve through the standard points.

Percentage absorbance = (B – blank OD)/(B_o – blank OD) where
B = OD of sample or standard and
B_o = OD of zero standard (total binding)

A. TYPICAL DATA

These standard curves are for demonstration only. A standard curve must be run with each assay.



B. SENSITIVITY

The minimum detectable concentration of PYY is 2.84 pg/ml.

C. DETECTION RANGE

0.1-1000 pg/ml

D. REPRODUCIBILITY

Intra-Assay: CV<10%
Inter-Assay: CV<15%

X. SPECIFICITY

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested: Ghrelin, Nesfatin, Angiotensin II, NPY and APC.

XI. REFERENCES

1. Murphy KG, Bloom SR (2006). "Gut hormones and the regulation of energy homeostasis". *Nature* **444** (7121): 854–9. PMID 17167473.
2. Batterham RL, Cohen MA, Ellis SM, Le Roux CW, Withers DJ, Frost GS, Ghatei MA, Bloom SR (2003). "Inhibition of food intake in obese subjects by peptide YY3-36". *The New England journal of medicine* **349** (10): 941–8. PMID 12954742.

XII. TROUBLESHOOTING GUIDE

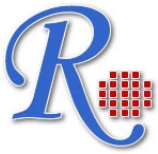
Problem	Cause	Solution
1. Poor standard curve	1. Inaccurate pipetting	1. Check pipettes
	2. Improper standard dilution	2. Ensure briefly spin the vial of Item C and dissolve the powder thoroughly by a gentle mix.
2. Low signal	1.Too brief incubation times	1. Ensure sufficient incubation time; assay procedure step 2 change to over night
	2. Inadequate reagent volumes or improper dilution	2. Check pipettes and ensure correct preparation
3. Large CV	1. Inaccurate pipetting	1. Check pipettes
4. High background	1. Plate is insufficiently washed	1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	2. Contaminated wash buffer	2. Make fresh wash buffer
5. Low sensitivity	1. Improper storage of the EIA kit	1. Store your standard at ≤ -20°C after receipt of the kit.
	2. Stop solution	2. Stop solution should be added to each well before measure

RayBio® EIA kits:

If you are interested in other EIA kits, please visit www.raybiotech.com for details.

Notes:

This product is for research use only.



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