Technical Bulletin

Peptide YY EIA Kit

for Serum, Culture Supernatant, and Cell Lysates

RAB0413

Storage Temperature: –20 °C

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 YY3-36.

There are two major forms of Peptide YY: PYY1-36 and PYY3-36 which is the most common form of circulating PYY. Peptide YY3-36 (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 PYY3-36 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 PYY3-36 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.

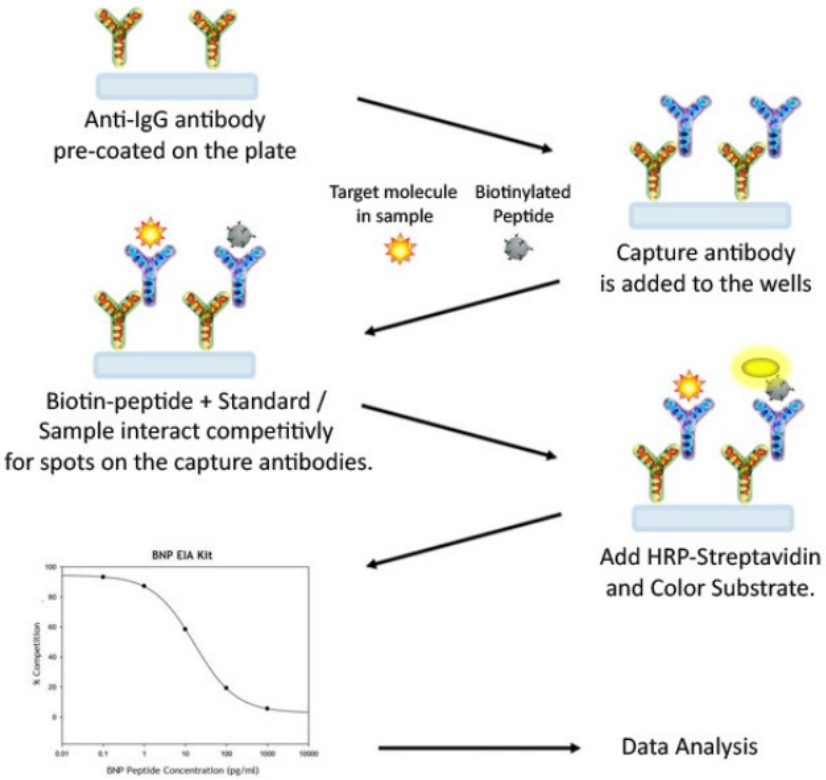
Studies have also shown that obese people secrete less PYY than non-obese people. The anorectic effect of PYY represents a possible anti-obesity therapy in the future.

Product Description

The Peptide YY (PYY) Enzyme Immunoassay (EIA) Kit is an *in vitro* quantitative assay for detecting Peptide YY based on the principle of competitive enzyme immunoassay.

In this assay, a biotinylated PYY peptide is spiked into the samples and standards. The samples and standards are then added to the plate, where the biotinylated PYY peptide competes with endogenous (unlabeled) PYY for binding to the anti-PYY antibody. After a wash step, any bound biotinylated PYY then interacts with horseradish peroxidase (HRP)-streptavidin, which catalyzes a color development reaction. The intensity of the colorimetric signal is directly proportional to the amount of captured biotinylated PYY peptide and inversely proportional to the amount of endogenous PYY in the 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.

How It Works



Storage

The entire kit may be stored at −20 °C to −80 °C for up to 6 months from the date of shipment. For extended storage, it is recommended to store at −80 °C. Avoid repeated freeze-thaw cycles. For prepared reagent storage, see table below.

Components

- EIA Microplate (Item A): 96 wells (12 strips x 8 wells) coated with secondary antibody. Store at 4 °C for up to a month after opening.
- Wash Buffer Concentrate (20X) (Item B): 25 mL of 20X concentrated solution. Store at 4 °C for up to a month after opening.
- Standard PYY Peptide (Item C): 2 vials of Lyophilized PYY Peptide. 1 vial is enough to run each standard in duplicate. Do not store and reuse.
- Anti-PYY Polyclonal Antibody (Item N): 2 vials of Lyophilized anti-PYY. Do not store and reuse.
- 5X Assay Diluent B (Item E): 15 mL of 5X concentrated buffer. Diluent for both standards and samples including serum, plasma, cell culture media or other sample types. Store at 4 °C for up to a month after opening.
- Biotinylated PYY Peptide (Item F): 2 vials of Lyophilized Biotinylated PYY Peptide, 1 vial is enough to assay the whole plate. Do not store and reuse.
- HRP-Streptavidin Concentrate (Item G): 600 µL 100X concentrated HRP-conjugated streptavidin. Do not store and reuse.
- Positive Control (Item M): 1 vial of Lyophilized Positive Control. Do not store and reuse.
- TMB One-Step Substrate Reagent (Item H): 12 mL of 3,3’,5,5’-tetramethylbenzidine (TMB) in buffer solution.
- Stop Solution (Item I): 8 mL of 0.2 M sulfuric acid.

Additional Materials Required (Not Provided)

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- 100 mL and 1 L graduated cylinders
- Absorbent paper
- Distilled or deionized water
- SigmaPlot® software (or other software which can perform four-parameter logistic regression models)
- Tubes to prepare standard or sample dilutions
- Orbital shaker
- Aluminum foil
- Plastic wrap

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Reagent Preparation

Keep kit reagents on ice during reagent preparation steps.

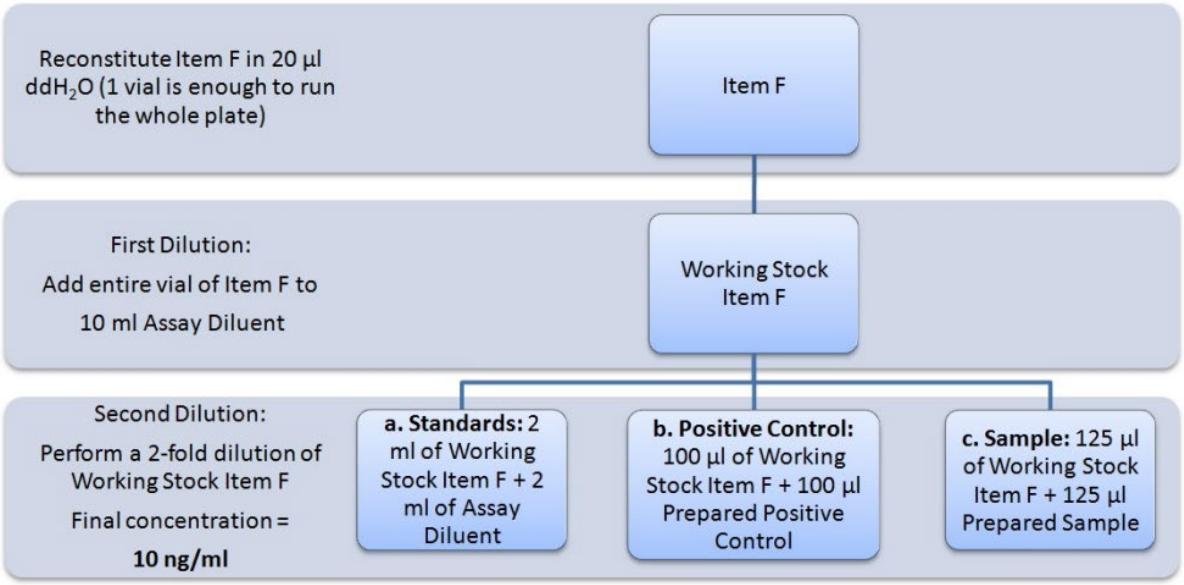
Preparation of Plate and Anti-PYY Antibody

1. Equilibrate plate to room temperature before opening the sealed pouch.
2. Label removable 8-well strips as appropriate for your experiment.
3. 5X Assay Diluent B (Item E) should be diluted 5-fold with deionized or distilled water.
4. Briefly centrifuge the anti-PYY antibody vial (Item N) and reconstitute with 55 µL of 1X Assay Diluent B to prepare the antibody concentrate. Pipette up and down to mix gently.
5. 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 Assay Procedure.

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

Preparation of Biotinylated PYY (Item F)

6. Briefly centrifuge the vial of Biotinylated PYY (Item F) and reconstitute with 20 µL of ddH_2O before use.
7. See the image below for proper preparation of Item F. Transfer the entire contents of the Item F vial into a tube containing 10 mL of 1X Assay Diluent B. This is your Working Stock of Item F. Pipette up and down to mix gently. The final concentration of biotinylated PYY will be 20 pg/mL.
    a. Second Dilution of Item F for Standards: Add 2 mL of Working Stock Item F to 2 mL of 1X Assay Diluent B. The final concentration of biotinylated PYY will be 10 pg/mL.
    b. Second Dilution of Item F for Positive Control: Add 100 µL of Working Stock Item F to 100 µL of the prepared Positive Control (Item M). (See section D for Positive Control preparation) The final concentration of biotinylated PYY will be 10 pg/mL.
    c. Second Dilution of Item F for samples: Add 125 µL of Working Stock Item F to 125 µL of prepared sample (see section E for sample preparation). This is a 2-fold dilution of your sample. The final concentration of biotinylated PYY will be 10 pg/mL.

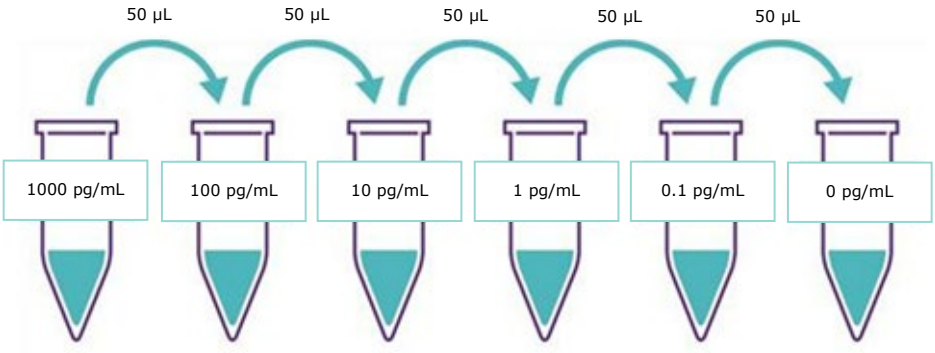


Preparation of Standards

8. Label 6 microtubes with the following concentrations: 1,000 pg/mL, 100 pg/mL, 10 pg/mL, 1 pg/mL, 0.1 pg/mL and 0 pg/mL. Pipette 450 µL of biotinylated PYY Item F working solution (prepared in step 7a) into each tube, except the 1,000 pg/mL (leave this one empty).

   Note: It is very important to make sure the concentration of the biotinylated PYY is 10 pg/mL in all standards.

9. Briefly centrifuge the vial of PYY Standard (Item C). Reconstitute with 10 µl of ddH2O and briefly vortex if desired. Pipette 8 µL of Item C and 792 µL of 10 pg/mL biotinylated PYY working solution (prepared in step 7a) into the tube labeled 1000 pg/mL. Mix thoroughly. This solution serves as the first standard (1000 pg/mL PYY standard, 10 pg/mL biotinylated PYY).
10. To make the 100 pg/mL standard, pipette 50 µL of the 1000 pg/mL PYY standard into the tube labeled 100 pg/mL. Mix thoroughly.
11. Repeat this step with each successive concentration, preparing a dilution series as shown in the illustration below. Each time, use 450 µL of biotinylated PYY and 50 µL of the prior concentration until the 0.1 pg/mL is reached. Mix each tube thoroughly before the next transfer.



Positive Control Preparation

12. Briefly centrifuge the Positive Control vial (Item M) and reconstitute with 100 µL of ddH_2O.
13. Refer to step 7b. This is a 2-fold dilution of the Positive Control. The final concentration of biotinylated PYY should still be 10 pg/mL.

    The Positive Control is a mouse serum sample that serves as a system control to verify that the kit components are working. The resulting OD will not be used in any calculations; if no positive competition is observed please contact Technical Support. The Positive Control may be diluted further if desired but be sure the final concentration of biotinylated PYY is 10 pg/mL.

Sample Preparation

14. If you wish to perform a 2-fold dilution of your sample, proceed to step 7c. If you wish to perform a higher dilution of your sample, dilute your sample with 1XAssay Diluent B before performing step 7c.

    EXAMPLE (to make a 4-fold dilution of sample):

    a. Dilute sample 2-fold (62.5 µL of sample + 62.5 µL of 1X Assay Diluent B.).
    b. Perform step 7c (125 µL of working solution Item F + 125 µL of sample prepared above).

    Note: The total volume is 250 µL, enough for duplicate wells on the microplate. It is very important to make sure the final concentration of the biotinylated PYY is 10 pg/mL.

    Note: Optimal sample dilution factors should be determined empirically, however you may reference below for recommended dilution factors for serum: Human = 2x Mouse = 2x Rat = 2x. If you have any questions regarding the recommend ended dilutions, please contact technical support.

Preparation of Wash Buffer and HRP

15. If Item B (20X Wash Concentrate) contains visible crystals, warm to room temperature and mix gently until dissolved.
16. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.
17. Briefly centrifuge the HRP-Streptavidin vial (Item G) before use.
18. Dilute the HRP-Streptavidin concentrate 100-fold with 1X Assay Diluent B.

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 of anti-PYY antibody (see Preparation, step 5) to each well. Incubate for 1.5 hours at room temperature with gentle shaking (1–2 cycles/sec) or incubate overnight at 4 °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 Section C), positive control (see Reagent Preparation Section D), and sample (see Reagent Preparation Section E) 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 °C.
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 18) to each well. Incubate with gentle shaking for 45 minutes at room temperature. 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.

Assay Procedure Summary

1. Prepare all reagents, samples and standards as instructed.
2. Add 100 µL anti-PYY 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.

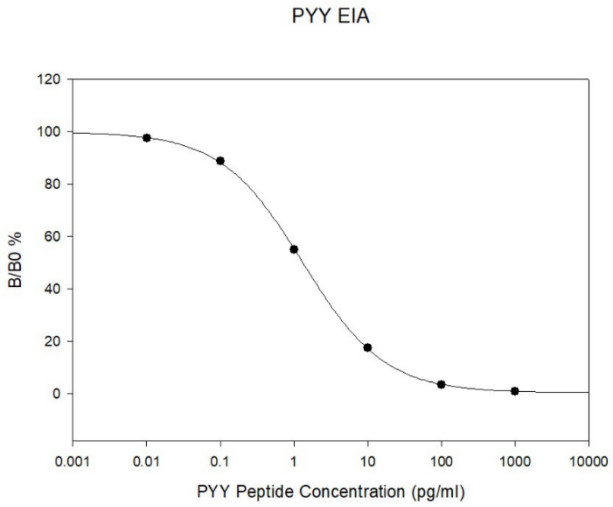
Calculation of Results

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

B_o = OD of zero standard (total binding)



Typical Data

Standard curves are for demonstration only. Standard curves must be run with each assay.

Sensitivity

The minimum detectable concentration of PYY is 5.6 pg/mL.

Standard Curve Range

0.1-1,000 pg/mL

Reproducibility

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

Specificity

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

This kit detects the 1-36 form of PYY.

Assay Diagram

Blank	Blank	SA1	SA1	SA9	SA9	SA17	SA17	SA25	SA25	SA33	SA33
Total Binding	Total Binding	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2
Standard 1	Standard 1	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2
Standard 1	Standard 1	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2
Standard 1	Standard 1	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2
Standard 1	Standard 1	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2
Standard 1	Standard 1	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2
Pos Control	Pos Control	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2	SA2

Key:

Blank = Buffer Only

Total Binding = Biotin-PYY Only

Standard 1 = 1000 pg/mL

Standard 2 = 100 pg/mL

Standard 3 = 10 pg/mL

Standard 4 = 1 pg/mL

Standard 5 = 0.1 pg/mL

Pos Control = Biotin with Item M

Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes.
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 2 may change to overnight.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation.
Large CV	Inaccurate pipetting	Check pipettes.
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer.
Low sensitivity	Improper storage of the ELISA kit	Store the standard at ≤ –20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light.
	Stop solution	Stop solution should be added to each well before measurement.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

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