



PYY ELISA Kit Instructions

For the quantitative determination of peptide YY (PYY)
in human serum and plasma

**Catalog #81500
96 Assays**

For research use only. Not for use in diagnostic procedures.

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A. Intended Use

The PYY ELISA kit is for the quantitative determination of PYY (both PYY (1-36) and PYY (3-36)) in human serum and plasma. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Peptide YY is a short (36-amino acid) peptide released by cells in the ileum, colon, and rectum in response to feeding. PYY shows an inhibitory action on contraction of the gastrointestinal tract and on secretion of pancreatic and gastric juice. PYY is believed to play a very important role in energy homeostasis by balancing food intake.

C. Principle of the Assay

The PYY ELISA kit is based on a competitive enzyme immunoassay using a combination of a highly specific antibody to human PYY and biotin-avidin affinity system.

D. Kit Storage

1. Upon receipt of the PYY ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

| Mark | Description | Amount |
|-------|---------------------------------------|----------------|
| MIC | Antibody-coated Microplate (96 wells) | 1 pack |
| STD | Standard | 1 vial (20 ng) |
| LA | Labeled Antigen | 1 vial |
| SAHRP | SA-HRP Solution | 1 x 12 mL |
| TMB | Enzyme Substrate Solution (TMB) | 1 x 12 mL |
| STOP | Stop Solution | 1 x 12 mL |
| BUF | Buffer Solution | 1 x 25 mL |
| WASH | Washing Solution (20X Concentrated) | 1 x 50 mL |
| SEAL | Adhesive seal | 3 sheets |

E.2. Materials required but not provided

- Micropipettes and disposable tips
- Distilled water
- Polypropylene microtubes
- Volumetric flasks
- Microplate shaker
- Microplate reader (capable of reading A₄₅₀ and A₆₃₀ values)

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.

G. Maximizing Kit Performance

1. Given the small sample volumes required (25 μ L), pipetting should be done as carefully as possible. A high quality 50 μ L or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each calibrator and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

An EDTA-2Na additive blood collection tube is recommended for plasma collection. If same blood is to be used to measure PYY (3-36) using another kit, add DPP-4 inhibitor to the collection tube immediately, yielding a 100 μ M final concentration. Serum and plasma samples should be used as soon as possible after collection. For later testing, samples should be aliquoted and stored at below -70°C . Avoid repeated freezing and thawing.

I. Assay Procedure

I.1. Preparation of reagents

1. Antibody-coated microplate
Provided as ready to use.
2. Standard
The standard is provided in lyophilized form (20 ng/vial). Working standards should be prepared immediately prior to use as described in Section I.2. The working standard concentrations are 0, 0.082, 0.247, 0.741, 2.222, 6.667, and 20 ng/mL. To prepare working standards, the lyophilized standard must be first reconstituted. The reconstituted 20 ng/mL standard once prepared is recommended to be stored frozen at below -70°C if not used all at once. The 20 ng/mL reconstituted standard should be aliquoted in appropriate volumes prior to being frozen. The reconstituted standard is stable for at least 2 months at below -70°C . The working standards should be discarded after each use.
3. Labeled Antigen
Reconstitute labeled antigen with 6 mL of Buffer Solution. Labeled antigen solution should be prepared immediately before use. The reconstituted labeled antigen solution once prepared is recommended to be stored frozen at below -70°C if not used all at once.
4. SA-HRP Solution
Provided as ready to use.

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5. Enzyme Substrate Solution (TMB)
Provided as ready to use.
6. Stop Solution
Provided as ready to use.
7. Buffer Solution
Provided as ready to use.
8. Wash Buffer (20X Concentrated)
The wash buffer has to be diluted 1:20 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 950 mL of distilled or deionized water.

I.2. Preparation of working standards

1. Reconstitute standard (20 ng/vial) with 1 mL of Buffer Solution (marked "BUF") and mix thoroughly, resulting in a 20 ng/mL reconstituted standard.
2. Dispense 0.2 mL of Buffer Solution into five polypropylene microtubes labeled 6.667, 2.222, 0.741, 0.247 and 0.082 ng/mL.
3. Dispense 0.1 mL of the 20 ng/mL standard into the 6.667 ng/mL microtube, and mix thoroughly.
4. Dispense 0.1 mL of the 6.667 ng/mL standard into the 2.222 ng/mL microtube, and mix thoroughly.
5. Dispense 0.1 mL of the 2.222 ng/mL standard into the 0.741 ng/mL microtube, and mix thoroughly.
6. Repeat this dilution scheme using the remaining microtubes.
7. Dispense 0.2 mL of Buffer Solution into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 20, 6.667, 2.222, 0.741, 0.247, 0.082 and 0 ng/mL.

Please note: Working standards should be prepared immediately prior to use.

I.3. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. To maximize stability, reconstituted standard and labeled antigen should be stored at below -70°C. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. Aspirate well contents and wash three times using 300 μ L of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
2. In each well, add 25 μ L of Buffer Solution.
3. In each well, add 50 μ L of sample or standard and mix well by repeated pipetting. Finally, add 25 μ L of Labeled Antigen solution to each well.
4. Cover the wells with adhesive seal and incubate the plate for 16-18 hours at 4°C without shaking.
5. Further incubate the plate for 40 minutes at room temperature.
6. Aspirate well contents and wash four times using 300 μ L of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
7. Add 100 μ L of the SA-HRP solution in each well.
8. Cover the wells with adhesive seal and incubate the plate for 2 hours at room temperature on a microplate shaker (shake at 100 rpm).
9. Aspirate well contents and wash four times using 300 μ L of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.

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10. Add 100 μ L of Enzyme Substrate Solution (TMB) in each well. Cover the wells with adhesive seal and incubate the plate for 30 mins in dark room at room temperature without shaking.
11. Stop the reaction by adding 100 μ L of Stop Solution to each well.
12. Measure absorbance within 30 minutes using a plate reader (measure A_{450} values and subtract A_{630} values).

I.4. Determining the PYY concentration

1. Using computer software, construct the PYY calibration curve by plotting the mean change in absorbance value for each calibrator (incl. blank) on the Y axis versus the corresponding PYY concentration on the X axis. A four or five parameter logistic curve fit is suitable for the evaluation.

Note: A calibration curve should be plotted every time the assay is performed.

2. PYY concentrations in the samples are interpolated using the calibration curve and mean absorbance values for each sample. The PYY concentration is expressed in ng/mL.

Note: Samples with high PYY concentrations (ie. fall above the range of the assay) should be further diluted with the Buffer Solution and rerun.

J. Performance characteristics

J.1. Assay range

The PYY ELISA Kit has an assay range from 0.082 – 20 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV < 20%.

J.3. Cross reactivity

The assay shows 100% cross reactivity to human PYY (3-36) and PYY (1-36). No cross reactivity with human NPY was observed.

Warranty

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