V.1

Crystal Chem

Mouse PYY ELISA Kit Instructions

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

Catalog #81501
96 Assays

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

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A. Intended Use

The Mouse PYY ELISA kit is for the quantitative determination of PYY (both PYY (1-36) and PYY (3-36)) in mouse 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 Mouse PYY ELISA kit is based on a competitive enzyme immunoassay. The 96-well plate is coated with goat anti-rabbit IgG, to which biotin labeled antigen, standard antigen or samples and rabbit anti-mouse PYY antibody are added for competitive immunoreaction. After incubation and plate washing, HRP labeled streptavidin (SA) is added to form HRP labeled SA-biotinylated antigen-antibody complex. Finally, HRP enzyme activity is determined by TMB and the concentration of mouse PYY is calculated.

D. Kit Storage

1. Upon receipt of the Mouse 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
LA	Labeled Antigen	1 vial
SA	Specific Antibody	1 x 8.5 mL
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
	Adhesive foil	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 A_{450} and A_{630} 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 µL), pipetting should be done as carefully as possible. A high quality 50 µL 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 (1 mg/mL) 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 µM 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 (12.5 ng/vial). Working standards should be prepared immediately prior to use as described in Section I.2. The working standard concentrations are 0, 0.15, 0.46, 1.39, 4.17, and 12.5 ng/mL. To prepare working standards, the lyophilized standard must be first reconstituted. The reconstituted 12.5 ng/mL standard once prepared is recommended to be stored frozen at below -70°C if not used all at once.The 12.5 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 7 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. Specific Antibody
   Provided as ready to use.
5. SA-HRP Solution
   Provided as ready to use.
6. Enzyme Substrate Solution (TMB)
   Provided as ready to use.
7. Stop Solution
   Provided as ready to use.
8. Buffer Solution
   Provided as ready to use.
9. 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 (12.5 ng/vial) with 1 mL of Buffer Solution (marked “BUF”) and mix thoroughly, resulting in a 12.5 ng/mL reconstituted standard.
2. Dispense 0.3 mL of the 12.5 ng/mL into a polypropylene microtube labeled 12.5 ng/mL.
3. Dispense 0.2 mL of Buffer Solution into five polypropylene microtubes labeled 4.17, 1.39, 0.46, and 0.15 ng/mL.
4. Dispense 0.1 mL of the 12.5 ng/mL standard into the 4.17 ng/mL microtube, and mix thoroughly.
5. Dispense 0.1 mL of the 4.17 ng/mL standard into the 1.39 ng/mL microtube, and mix thoroughly.
6. Dispense 0.1 mL of the 1.39 ng/mL standard into the 0.46 ng/mL microtube, and mix thoroughly.
7. Repeat this dilution scheme using the remaining microtubes.
8. Dispense 0.2 mL of Buffer Solution into one polypropylene microtube labeled 0 ng/mL. You should now have working standards of 12.5, 4.17, 1.39, 0.46, 0.15, 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 350 µL 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 50 µL of Labeled Antigen solution.
3. In each well, add 25 µL of sample or standard and mix well by repeated pipetting.
4. In each well, add 75 µL of specific antibody solution and mix well by repeated pipetting.

5. Cover the wells with adhesive foil and incubate the plate for 18 hours (± 1 hour) at 4°C without shaking. Further incubate the plate for 30 minutes at room temperature on a microplate shaker (shake at 100-150 rpm).
6. Aspirate well contents and wash five times using 350 µL 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 µL of the SA-HRP solution in each well.
8. Cover the wells with adhesive foil and incubate the plate for 1 hour at room temperature on a microplate shaker (shake at 100-150 rpm).
9. Aspirate well contents and wash five times using 350 µL of Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
10. Add 100 µL of Enzyme Substrate Solution (TMB) in each well. Cover the wells with adhesive foil and incubate the plate for 30 mins in dark room at room temperature without shaking.
11. Stop the reaction by adding 100 µL 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 higher-grade polynomial or four parametric logistic (4-PL) curve fit are suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Mouse 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 mouse 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 Mouse PYY ELISA Kit has an assay range from 0.15 – 12.5 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 mouse PYY (3-36) and 115% to mouse PYY (1-36). No cross reactivity with mouse NPY, GLP-1 (7-36)-NH2, GLP-1 (1-37), and GLP-2 were observed.

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