Peptide YY ELISA Kit

Enzyme Immunoassay for the quantification of Human Peptide YY (3-36) and Human Peptide YY (1-36) in serum and plasma samples.

Catalog number: ARG82774

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MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

Peptide YY (PYY) also known as peptide tyrosine tyrosine is a peptide that in humans is encoded by the PYY gene. Peptide YY is a short (36-amino acid) peptide released from cells in the ileum and colon in response to feeding. In the blood, gut, and other elements of periphery, PYY acts to reduce appetite; similarly, when injected directly into the central nervous system, PYY is also anorexigenic, i.e., it reduces appetite.

Dietary fibers from fruits, vegetables, and whole grains, consumed, increase the speed of transit of intestinal chyme into the ileum, to raise PYY3-36, and induce satiety. Peptide YY can be produced as the result of enzymatic breakdown of crude fish proteins and ingested as a food product.

Peptide YY is related to the pancreatic peptide family by having 18 of its 36 amino acids located in the same positions as pancreatic peptide. The two major forms of peptide YY are PYY1-36 and PYY3-36, which have PP fold structural motifs. However, the most common form of circulating PYY immunoreactivity is PYY3-36, which binds to the Y2 receptor (Y2R) of the Y family of receptors. Peptide YY3-36 (PYY) is a linear polypeptide consisting of 34 amino acids with structural homology to NPY and pancreatic polypeptide. PYY exerts its action through NPY receptors; it 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 a meal. Research has also indicated PYY may be useful in removing aluminium accumulated in the brain.

Obese patients undergoing gastric bypass showed marked metabolic adaptations, resulting in frequent diabetes remission 1 year later. When the

confounding of calorie restriction is factored out, β-cell function improves rapidly, very possibly under the influence of enhanced GLP-1 responsiveness. Insulin sensitivity improves in proportion to weight loss, with a possible involvement of PYY. [wikipedia]

PRINCIPLE OF THE ASSAY

This is an Enzyme Immunoassay for the quantification Peptide YY in human serum and EDTA-plasma samples. (This kit is designed for serum and plasma samples but it may be used for other samples when the expression level is fall within the linear range.) This assay employs the competitive quantitative enzyme immunoassay technique. A secondary antibody has been pre-coated onto a microtiter plate. The secondary antibody can bind to the Fc fragment of the primary antibody which recognizes Peptide YY. The primary antibodies in the kit will be competitively bound by biotinylated-Peptide YY peptides and Peptide YY peptides in standards or targeted Peptide YY peptides in samples. The wells are washed and then incubated with Streptavidin-HRP reagent. The biotinylated peptide interacts with streptavidin-horseradish peroxidase to form a complex. After washing away any unbound Streptavidin-HRP reagent, a substrate solution (TMB) is added to the wells and color develops in inverse-proportion to the amount of Peptide YY peptide present in the samples. The color development is stopped by the addition of STOP solution and the intensity of the color is measured at a wavelength of 450nm±2 nm. The concentration of Peptide YY peptide in the sample is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

Component	Quantity	Storage information
Secondary antibody coated microplate	12 x 8 wells	4°C
Standard (Peptide YY 3-36)	1 vial	4°C
Biotinylated peptide	1 vial	4°C
20X Wash Buffer	50 ml	4°C
Primary antibody	1 vial	4°C
1000X Streptavidin-HRP conjugate	30 µl	4°C
Diluent Buffer	23 ml (Ready-to-use)	4°C
TMB substrate	12 ml (Ready-to-use)	4°C (Protect from light)
STOP solution (2N HCl)	15 ml (Ready-to-use)	4°C
Positive Controls (Accept. Range dependent on lot#)	2 vials	4°C
Plate sealer	3 pieces	Room Temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes, Multi-channel pipette and pipette tips
- Deionized or distilled water
- Solution reservoir
- Microplate shaker (300-400rpm)
- Aprotinin (enzyme inhibitor)
- Automated microplate washer (optional)

TECHNICAL HINTS AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- The kit should be equilibrated to room temperature (20-23°C) before opening any vials and starting the assay.
- Before opening any Eppendorf tubes for reconstitution, briefly centrifuge at ~3,000rpm for 5 seconds to ensure that all the lyophilized material is at the bottom of the tube.
- If crystals are observed in the 20X Wash buffer, warm to RT or 37°C until the crystals are completely dissolved.
- Ensure complete reconstitution and dilution of reagents prior to use.
- It is highly recommended that all solutions be used as soon as possible after reconstitution.
- Unused microplate strips should be placed back in the foil pouch with a desiccant and stored at 4°C. Do not allow moisture to enter the wells.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Each time a new tip is used, make sure the tip is secure and free of air bubbles. For better intra-assay variation, aspirate and expel a reagent or sample back into the container a few times prior to loading.
- Avoid submerging the whole tip into reagents because droplets can accumulate at the end of the tip causing an excess of reagent to be loaded into the well. This can lead to poor results.

- For optimal results, an orbital plate shaker capable of 300-400 rpm is recommended for all incubations.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. This kit is designed for serum and plasma samples but it may be used for other samples when the expression level is fall within the linear range.

Serum- Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1600 x g at 4°C. Collect serum and assay immediately or aliquot and store samples at ≤ -80 °C up to 1 month. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA as an anticoagulant. Centrifuge for 15 minutes at 4°C at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -80 °C up to 1 month. Avoid repeated freeze-thaw cycles.

Note: We recommended add Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.6 TIU or 100 µl of Aprotinin per mL of sample solution.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash buffer. Store the diluted 1X Wash Buffer at 4°C. If crystals appear in 20X Wash Buffer, warm the buffer in 37°C water bath for 30 minutes or until crystals disappear. Mix well before use.
- Primary antibody: Reconstitute the Primary antibody vial with 1 ml of 1X Wash Buffer to yield an antibody stock solution. Allow it to sit for 5-10 minutes to completely dissolve, mix well and keep the stock solution at 4°C before use. Store the antibody stock solution at 4°C for up to 4 days. For long-term storage, it is recommended to aliquot & store the stock at -20°C or -70°C for up to 2 months. Avoid repeated freeze-thaw cycles.

  Dilute the antibody stock solution 1:4 with Diluent Buffer to yield 1X Primary antibody solution, mix thoroughly. (e.g. 1 ml of antibody stock solution + 4 ml of Diluent Buffer)
- Biotinylated peptide: Reconstitute the Biotinylated peptide vial with 1 ml of 1X Wash Buffer to yield a biotinylated peptide stock solution. Allow it to sit for 5-10 minutes to completely dissolve, mix well and keep the stock solution at 4°C before use. Store the biotinylated peptide stock at 4°C for up to 4 days. For long-term storage, it is recommended to aliquot & store at -20°C or -70°C for up to 2 months. Avoid repeated freeze-thaw cycles. It is recommended that peptide should only be frozen-thawed once.

  Prior to use, mix thoroughly before dilution, dilute the biotinylated peptide stock 1:4 with Diluent Buffer to yield biotinylated peptide

working solution. Allow it to sit for at least 5 minutes to completely dissolve, mix thoroughly. (e.g. 1 ml of biotinylated peptide stock+ 4 ml of Diluent Buffer)

- **Positive control**: Centrifuge and reconstitute the Positive control vial with 200 µl of 1X Wash Buffer. Allow it to sit for 5-10 minutes to completely dissolve, mix well and keep rehydrated solution at 4°C before use. Store the reconstituted control at 4°C for up to 4 days. For long-term storage, it is recommended to aliquot & store at -20°C or -70°C for up to 2 months. Avoid repeated freeze-thaw cycles. It is recommended that control should only be frozen-thawed once. (acceptable range dependent on lot#)
- **1X Streptavidin-HRP conjugate:** 1X Streptavidin-HRP conjugate working solution should be prepared freshly before use. Centrifuge 1000X Streptavidin-HRP conjugate briefly and add 12µl of Streptavidin-HRP to 12ml 1X wash buffer to make a 1X Streptavidin-HRP working solution. Vortex thoroughly.
- **Sample:** Dilute serum/plasma sample 1:1 with Diluent Buffer. Vortex and centrifuge before use. (Dilution factor =2) It is highly recommended that normal plasma samples be used in comparison with patient plasma samples to establish a baseline value. If further dilutions are required, dilute serum/plasma samples in Diluent Buffer. For the calculation of the concentrations this dilution factor has to be taken into account.

- **Standard peptide**: Centrifuge and reconstitute the standard with 1 ml of 1X Wash buffer and vortex. The concentration of this stock solution is 1000 ng/ml. Allow the solution to sit for at least 10 minutes at room temperature to completely dissolve. Store the reconstituted standard at 4°C for up to 4 days. For long-term storage, it is recommended to aliquot & store at -20°C or -70°C for up to 2 months. Avoid repeated freeze-thaw cycles. It is recommended that standard should only be frozen-thawed once. Dilute peptide standard solutions with Diluent Buffer to 100 ng/ml, 10 ng/ml, 1 ng/ml, 0.1 ng/ml, 0.01 ng/ml as follows:

The example of the dilution of standards

Standard No.	Standard Conc. (ng/ml)	Diluent Buffer (µl)	Standard (µl)
Stock	1000	-	-
S1	100	450	50 µl of Stock
S2	10	450	50 µl of S1
S3	1	450	50 µl of S2
S4	0.1	450	50 µl of S3
S5	0.01	450	50 µl of S4
S0 (Total binding)	0	450	0

ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-23°C) 30 minutes before opening and starting the assay. Standards, samples, control and blank should be assayed in duplicates.

1. Remove excess microtiter strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add **50 µl of Diluent Buffer** as Total Binding (S0, zero standard). Two empty wells should be left as blank.
3. Add **50 µl of prediluted peptide standards** (add from S5 to S1), **50 µl positive controls** or **50 µl diluted samples** into corresponding wells. It is advisable to assay each condition in duplicates.
4. Add **25 µl of 1X primary antibody** into each well **except the Blank wells**.
5. Add **25 µl of Biotinylated peptide working solution** into each well **except the Blank wells**. It is not recommended to use a multi-channel pipette to load the primary antibody and biotinylated peptide.
6. Seal the microtiter plate with plate sealer. Incubate for **2 hours at RT (20-23°C).** Orbital shaking on a microplate shaker at 300-400 rpm is recommended.
7. Prepare 1X Streptavidin-HRP conjugate working solution: Mix and centrifuge Streptavidin-HRP concentrate vial (3,000-5,000 rpm for 5 seconds) before use. Pipette 12 µl of Streptavidin-HRP concentrate into 12 ml of 1X Wash Buffer to make a Streptavidin-HRP working solution. Vortex thoroughly. Prepare freshly.
8. Remove sealer from plate.
9. Aspirate each well and wash, repeating the process 3 times for a **total 4**

washes. Wash by filling each well with **1× Wash Buffer (350 µl)** using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.

10. Add **100 µl of diluted (1X) Streptavidin-HRP** working solution into **each well**.
11. Reseal the plate with sealer. Incubate for **1 hour at RT.** Orbital shaking on a microplate shaker at 300-400 rpm is recommended.
12. Remove sealer from plate. **Wash** as according to step 9.
13. Add **100 µl of TMB substrate** solution into **each well**.
14. Reseal the plate with sealer. Incubate for **1 hour at RT in dark.** Orbital shaking on a microplate shaker at 300-400 rpm is recommended.
15. Remove sealer from plate. (**DO NOT** wash or discard the contents of the wells)
16. Add **100 µl of STOP solution (2N HCl)** into **each wells** to stop the reaction. Gently tap the plate to ensure thorough mixing. The color of the solution should change from blue to yellow.
17. Read the OD with a microplate reader at **450 nm** immediately. It is recommended that the wells be read within 20 minutes after adding the Stop Solution.

CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards, controls and patient samples.

2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

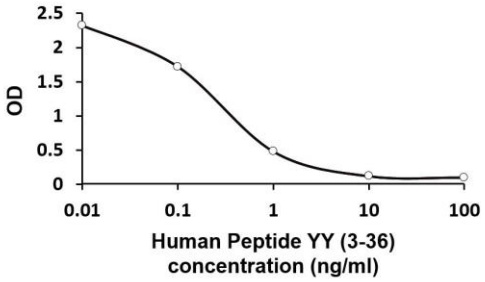
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.

4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.

5. If samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors. Because Serum/plasma samples have been diluted prior to the assay, the measured concentration must be multiplied by their respective dilution factors (e.g. 1ng/ml (from standard curve) x 2 (dilution factor) = 2ng/ml).

EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



QUALITY ASSURANCE

Sensitivity

The standard of Peptide YY peptide ranged from 0-100 ng/ml.

The mean MDD was 0.079 ng/ml.

Linear Range

0.079 – 0.88 ng/ml

Precision:

Intra-assay: < 10%

Inter-assay: < 15%

Cross Reactivity

The cross reactivity ratio of the tested peptide as the table:

Peptide	Cross Reactivity (%)
Human Peptide YY (3-36)	100
Human Peptide YY	100
Mouse, Rat, Pig and Dog Peptide YY	26
Mouse, Rat, Pig and Dog Peptide YY (3-36)	10
Pig Neuropeptide Y	11
Human Pancreatic Polypeptide	0
Human Insulin	0
Human Glucagon	0
Human Amylin Amide	0
Substance P	0