# Human PYY (Total)

# 125 Tubes

# Cat. # PYYT-66HK

# HUMAN PYY (Total) RIA KIT
# 125 TUBES (Cat. # PYYT-66HK)




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## I. INTENDED USE

Peptide YY (P-YY), a novel 36 amino-acid amidated hormone is a component of the complex neuroendocrine control process. This gut hormone (fragment 3-36) when infused into subjects has been shown to reduce food intake in normal weight and obese individuals. PYY infusion also reduced the plasma levels of the hunger-promoting hormone ghrelin. PYY levels have been shown to drop pre-meal and then increase post prandially. In circulation, PYY exists at least in two molecular forms: 1-36 and 3-36.

EMD Millipore's PYY (Total) Radioimmunoassay (RIA) Kit utilizes an antibody, which recognizes both the 1-36 and 3-36 forms of Human PYY. Sensitivity of 10 pg/mL can easily be achieved when using a 100 µL serum or plasma sample in a two-day, disequilibrium assay (400 µL Total Volume). ***For Research Use Only. Not for Use in Diagnostic Procedures.***

## II. PRINCIPLES OF PROCEDURE

In radioimmunoassay, a fixed concentration of labeled tracer antigen is incubated with a constant dilution of antiserum such that the concentration of antigen binding sites on the antibody is limited, for example, only 40%-50% of the total tracer concentration may be bound by antibody. If unlabeled antigen is added to this system, there is competition between labeled tracer and unlabeled antigen for the limited and constant number of binding sites on the antibody. Thus, the amount of tracer bound to antibody will decrease as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free tracer and counting one or the other, or both fractions. A standard curve is set up with increasing concentrations of standard unlabeled antigen and from this curve the amount of antigen in unknown samples can be calculated. Thus, the four basic necessities for a radioimmunoassay system are: a specific antiserum to the antigen to be measured, the availability of a radioactive labeled form of the antigen, a method whereby antibody-bound tracer can be separated from the unbound tracer, and finally, an instrument to count radioactivity.

The EMD Millipore PYY (Total) assay utilizes $^{125}$I-labeled PYY and a PYY antiserum to determine the level of total PYY in serum, plasma or tissue culture media by the double antibody/PEG technique.

## III. REAGENTS SUPPLIED

Each kit is sufficient to run 125 tubes and contains the following reagents.

**A. Assay Buffer**
A buffer containing BSA and 0.08% sodium azide
Quantity: 2 bottles, 25 mL/vial
Preparation: Ready to use

**B. Human PYY (Total) Antibody**
Guinea Pig anti-PYY Serum in Assay Buffer
Quantity: 13 mL/vial
Preparation: Ready to use

**C. $^{125}$I-Human PYY**
$^{125}$I-PYY Label, HPLC purified (specific activity 302 µCi/µg)
Lyophilized for stability. Freshly iodinated label contains <1.5 µCi (56 kBq), calibrated to the 1st Monday of each month.
Quantity: 13.5 mL/vial upon hydration
Preparation: Contents Lyophilized. Hydrate with 13.5 mL of Assay Buffer. Allow to set at room temperature for 30 minutes, with occasional gentle mixing.

**D. Human PYY Standards**
Synthetic lyophilized PYY in Assay Buffer
Lyophilized for stability.
Quantity: 2 mL/vial upon hydration
Preparation: Contents Lyophilized. Hydrate with 2 mL distilled or deionized water. The actual concentration of PYY present in the vial will be lot-dependent. Please refer to the analysis sheet for exact PYY concentration present in a specific lot.

**E. Human PYY (Total) Quality Controls 1 & 2**
Synthetic lyophilized PYY in Assay Buffer.
Lyophilized for stability.
Quantity: 1 mL/vial upon hydration
Preparation: Contents Lyophilized. Hydrate with 1 mL distilled or deionized water.

**F. Guinea Pig Carrier**
Normal Guinea Pig Serum
Quantity: 2 mL/vial
Preparation: Ready to use

**G. Precipitating Reagent**
Goat anti-Guinea Pig IgG Serum, 3% PEG and 0.05% Triton X-100 in 0.05M Phosphosaline, 0.025M EDTA, 0.08% Sodium Azide
Quantity: 130 mL/vial
Preparation: Ready to use; chill to 4°C.

## IV. STORAGE AND STABILITY

Upon receipt, unused kit may be stored between 2 and 8°C for short term storage. For prolonged storage (>2 weeks), freeze unused kit at ≤ -20°C. Lyophilized components upon hydration should be stored at ≤-20°C immediately after use, or discarded. Avoid multiple (>5) freeze/thaw cycles. Refer to date on bottle for expiration when stored at ≤ -20°C. Do not mix reagents from different kits unless they have the same lot number and are unopened.

## V. REAGENT PRECAUTIONS

### A. Radioactive Materials

This radioactive material may be received, acquired, possessed and used only by research personnel or clinical laboratories for in vitro research tests not involving internal or external administration of the material, or the radiation there from to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to the regulations of the U. S. Nuclear Regulatory Commission (NRC) or of a State with which the Commission has entered into an agreement for the exercise of regulatory authority.

The following are suggested general rules for the safe use of radioactive material. The customer's Radiation Safety Officer is ultimately responsible for the safe handling and use of radioactive material.

1. Wear appropriate personal devices at all times while in areas where radioactive materials are used or stored.
2. Wear laboratory coats, disposable gloves, and other protective clothing at all times.
3. Monitor hands, shoes, and clothing and immediate area surrounding the workstation for contamination after each procedure and before leaving the area.
4. Do not eat, drink, or smoke in any area where radioactive materials are stored or used.
5. Never pipette radioactive material by mouth.
6. Dispose of radioactive waste in accordance with NRC rules and regulations.
7. Avoid contaminating objects such as telephones, light switches, doorknobs, etc.
8. Use absorbent pads for containing and easily disposing of small amounts of contamination.
9. Wipe up all spills immediately and thoroughly and dispose of the contaminated materials as radioactive waste. Inform Radiation Safety Officer.

### B. Sodium Azide

Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

**Note: See Full Labels of Hazardous components on next page.**

**Full labels of hazardous components in this kit:**

| Ingredient, Cat # | | Full Label | |
|---|---|---|---|
| Human PYY Standard | 8066-K |  | **Danger.** Harmful if swallowed. May damage fertility or the unborn child. Toxic to aquatic life with long lasting effects. Obtain special instructions before use. Avoid release to the environment. IF exposed or concerned: Get medical advice/ attention. |
| Precipitating Reagent | PR-UVHK |  | **Warning.** Causes serious eye irritation. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. |
| Human PYY (Total) Quality Controls 1 & 2 | 6066-K |  | **Danger.** Harmful if swallowed. May damage fertility or the unborn child. Toxic to aquatic life with long lasting effects. Obtain special instructions before use. Avoid release to the environment. IF exposed or concerned: Get medical advice/ attention. |

**Full Hazardous Label (continued)**

| Ingredient, Cat # | | Full Label | |
|---|---|---|---|
| $^{125}$I-Human PYY Tracer | 9066-HK |  | **Danger.** Harmful if swallowed. May damage fertility or the unborn child. Toxic to aquatic life with long lasting effects. Obtain special instructions before use. Avoid release to the environment. IF exposed or concerned: Get medical advice/ attention. |

## VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Borosilicate glass tubes, 12 x 75 mm. (NOTE: Polypropylene or polystyrene tubes may be used if the pellet formation is acceptably stable.)
2. 100 µL pipette with disposable tips
3. 10 µL, 100 µL & 1.0 mL repeating dispenser
4. Refrigerated swing bucket centrifuge capable of developing 2,000 - 3,000 xg. (Use of fixed-angle buckets is not recommended.)
5. Absorbent paper
6. Vortex mixer
7. Refrigerator
8. Gamma Counter
9. Aprotinin (recommended in SPECIMEN COLLECTION AND STORAGE section)

## VII. SPECIMEN COLLECTION AND STORAGE

**Note: Samples should be processed as quickly as possible and kept on ice to retard the breakdown of PYY. We recommend treatment of the blood with Aprotinin at a final concentration of 500 KIU/mL of blood. For total PYY measurement using this RIA, DPP-IV inhibitor is not required. However for future measurement of 3-36 PYY with the same set of samples we suggest the addition of 10 µL of DPP-IV inhibitor per one mL of blood along with the aprotinin.**

1. A maximum of 100 µL per assay tube of serum or plasma should be used. Tissue culture and other media may also be used.

2. Care must be taken when using heparin as an anticoagulant, since excess will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

3. For longer storage, specimens should be aliquot and stored at ≤ - 20ºC or below. Multiple freeze/thaw cycles should be avoided.

4. Avoid using samples with gross hemolysis or lipemia.

## VIII. ASSAY PROCEDURE

For optimal results, accurate pipetting and adherence to the protocol are recommended.

### A. PYY Standard Preparation

Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the PYY Standard with **2 mL** distilled or deionized water into the glass vial to give a concentration prescribed in the analysis sheet. Invert and mix gently, let sit for five minutes or until completely dissolved then mix well.

Label seven glass tubes 1, 2, 3, 4, 5, 6 and 7. Add 0.5 mL Assay Buffer to each of the seven tubes. Prepare serial dilutions by adding 0.5 mL of the reconstituted standard to tube 1, mix well and transfer 0.5 mL of tube 1 to tube 2, mix well and transfer 0.5 mL of tube 2 to tube 3, mix well and transfer 0.5 mL of tube 3 to tube 4, mix well and transfer 0.5 mL of tube 4 to tube 5, mix well and transfer 0.5 mL of tube 5 to tube 6, mix well and transfer 0.5 mL of tube 6 to tube 7 and mix well.

Note: Do not use plastic tubes; glass tubes must be used. Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Standard before dispensing. Unused portions of standard should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

| Standard Concentration pg/mL | Volume of Deionized Water to Add | Volume of Standard to Add |
|---|---|---|
| X (Refer to analysis sheet for exact concentration) | 2 mL | 0 |

| Tube # | Standard Concentration pg/mL | Volume of Assay Buffer to Add | Volume of Standard to Add |
|---|---|---|---|
| 1 | X/2 | 0.5 mL | 0.5 mL of reconstituted standard |
| 2 | X/4 | 0.5 mL | 0.5 mL of tube 1 |
| 3 | X/8 | 0.5 mL | 0.5 mL of tube 2 |
| 4 | X/16 | 0.5 mL | 0.5 mL of tube 3 |
| 5 | X/32 | 0.5 mL | 0.5 mL of tube 4 |
| 6 | X/64 | 0.5 mL | 0.5 mL of tube 5 |
| 7 | X/128 | 0.5 mL | 0.5 mL of tube 6 |

### B. PYY Quality Control 1 and 2 Preparation

1. Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the PYY Quality Control 1 and Quality Control 2 with **1 mL** distilled or deionized water into the glass vials. Invert and mix gently, let sit for five minutes or until completely dissolved then mix well.

Note: For exact concentration of Quality Control 1 and 2, refer to Analysis Sheet. Unused portions of Quality Controls should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

**Day One**

1. Pipette 300 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4). Pipette 200 µL of Assay Buffer in the Reference (Bo) tubes (5-6). Pipette 100 µL of Assay Buffer to tubes seven through the end of the assay.
2. Pipette 100 µL of Standards and Quality Controls in duplicate (see assay flow chart).
3. Pipette 100 µL of each sample in duplicate. (NOTE: Smaller volumes of sample may be used when PYY concentrations are anticipated to be elevated or when sample size is limited. Additional Assay Buffer should be added to compensate for the difference so that the volume is equivalent to 100 µL (e.g., when using 50 µL of sample, add 50 µL of Assay Buffer). Refer to Section IX for calculation modification.
4. Pipette 100 µL of PYY Antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
5. Vortex, cover, and incubate overnight (20-24 hours) at 4ºC.

## VIII. ASSAY PROCEDURE (continued)

### Day Two

6. Hydrate the $^{125}$I-PYY tracer with 13.5 mL of Assay Buffer and gently mix. Pipette 100 µL of $^{125}$I-PYY to all tubes.
7. Vortex, cover and incubate overnight (22-24 hours) at 4ºC.

### Day Three

8. Add 10 µL of Guinea Pig Carrier to all tubes except Total Count tubes (1-2).
9. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes except Total Count tubes (1-2).
10. Vortex and incubate 20 minutes at 4°C.
11. Centrifuge, at 4°C, for 20 minutes at 2,000-3,000 xg. Note: If less than 2,000 xg is used, the time of centrifugation must be increased to obtain a firm pellet (e.g. 40 minutes). Multiple centrifuge runs within an assay must be consistent. Conversion of rpm to xg:

    $xg = (1.12 \times 10^{-5}) (r) (rpm)^2$

    r = radial distance in cm (from axis of rotation to the bottom of the tube)

    rpm = revolutions per minute

12. Immediately decant supernatant from all centrifuged tubes except Total Count tubes (1-2). Drain tubes for 15-60 seconds (be consistent between racks), blot excess liquid from lip of tubes and count pellet using the gamma counter according to the manufacturer's instructions.

## VIII. ASSAY PROCEDURE (continued)

### Assay Procedure Flow Chart

| Day One | | | | | Day Two | | Day Three | | |
|---|---|---|---|---|---|---|---|---|---|
| Set-up | Step 1 | Step 2&3 | Step 4 | Step 5 | Step 6 | Step 7 | Step 8 | Step 9 | Steps 10-12 |
| Tube Number | Add Assay Buffer | Add Standard/QC/ Sample | Add PYY Antibody | **Vortex, Cover, and Incubate 20-24 hrs at 4°C** | Add I-125 PYY Tracer | **Vortex, Cover and Incubate 22-24 hrs at 4°C** | Add Guinea Pig Carrier | Add Precipitating Reagent | **Incubate 20 min. at 4°C, Centrifuge at 4°C for 20 min Decant and Count** |
| 1,2 | - | - | - | | 100 µL | | - | | |
| 3,4 | 300 µl | - | - | | 100 µL | | 10 µL | 1.0 mL | |
| 5,6 | 200 µL | - | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 7,8 | 100 µL | 100 µL of tube 7 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 9,10 | 100 µL | 100 µL of tube 6 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 11,12 | 100 µL | 100 µL of tube 5 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 13,14 | 100 µL | 100 µL of tube 4 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 15,16 | 100 µL | 100 µL of tube 3 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 17,18 | 100 µL | 100 µL of tube 2 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 19,20 | 100 µL | 100 µL of tube 1 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 21,22 | 100 µL | 100 µL of reconstituted standard | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 23,24 | 100 µL | 100 µL of QC 1 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 25,26 | 100 µL | 100 µL of QC 2 | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |
| 27,n | 100 µL | 100 µL of unknown | 100 µL | | 100 µL | | 10 µL | 1.0 mL | |

## IX. CALCULATIONS

### A. Explanation

The calculations for PYY can be automatically performed by most gamma counters possessing data reduction capabilities or by independent treatment of the raw data using a commercially available software package. Choose weighted 4-parameter or weighted log/logit for the mathematical treatment of the data. [NOTE: Be certain the procedure used subtracts the NSB counts from each average count, except Total Counts, prior to final data reduction.]

### B. Manual Calculation

1. Average duplicate counts for Total Count tubes (1-2), NSB tubes (3-4), Total Binding tubes (reference, Bo) (5-6), and all duplicate tubes for standards and samples to the end of the assay.
2. Subtract the average NSB counts from each average count (except for Total Counts). These counts are used in the following calculations.
3. Calculate the percentage of tracer bound

   (Total Binding Counts/Total Counts) X 100

   This should be 35-50%.
4. Calculate the percentage of total binding (%B/Bo) for each standard and sample

   %B/Bo = (Sample or Standard/Total Binding) X 100
5. Plot the % B/Bo for each standard on the y-axis and the known concentration of the standard on the x-axis using log-log graph paper.
6. Construct the reference curve by joining the points with a smooth curve.
7. Determine the pg/mL of PYY in the unknown samples and controls by interpolation of the reference curve.

   [NOTE: When sample volumes assayed differ from 100 µl, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 50 µl of sample is used, then calculated data must be multiplied by 2).]

## X. INTERPRETATION

### A. Acceptance Criteria

1. The run will be considered accepted when all Quality Control values fall within the calculated Quality Control Range; if any QC's fall outside the control range, review results with the supervisor.
2. If the difference between duplicate results of a sample is >10% CV, repeat the sample.

## XI. ASSAY CHARACTERISTICS

### A. Sensitivity

The lowest level of PYY that can be detected by this assay is 10 pg/mL when using a 100µL sample size.

### B. Performance

The following parameters of assay performance are expressed as Mean ± Standard Deviation.

$ED_{80}$ = 36 ± 5
$ED_{50}$ = 103 ± 12
$ED_{20}$ = 300 ± 38

### C. Specificity

The specificity (also known as selectivity) of an analytical test is its ability to selectively measure the analyte in the presence of other like components in the sample matrix.

| PYY RIA Crossreactivity | |
|---|---|
| PYY 1-36 human | 100% |
| PYY 3-36 human | 100% |
| [Pro34] PYY | 100% |
| [Leu31, Pro34] PYY | 100% |
| Rat/Porcine PYY 1-36 | <0.1% |
| Rat/Porcine PYY 3-36 | <0.1% |
| HPPP | <0.1% |
| NPY | <0.1% |
| Human Leptin | * |
| Glucagon | * |
| Human Ghrelin | * |
| Human Insulin | * |
| GLP-1 | * |

*-Not detectable

### D. Precision

Within and Between Assay Variation

| Sample no. | Mean pg/mL | Within %CV | Between %CV |
|---|---|---|---|
| 1 | 82.7 | 9.4 | 8.5 |
| 2 | 111.1 | 2.9 | 7.1 |
| 3 | 542.6 | 3.6 | 5.5 |

Within and between assay variations were performed on three human plasma samples containing varying concentrations of Human PYY. Data (mean and %CV) shown are from one assay with eight duplicate determinations of each plasma sample for intra-assay precision. For inter-assay precision, data are generated using eight separate assays run for the three samples in duplicate.

## XI. ASSAY CHARACTERISTICS (continued)

### E. Recovery

Spike and Recovery of PYY in Human Plasma

| Sample No. | PYY added pg/mL | %Recovery |
|---|---|---|
| 1 | 40 | 111 |
| 2 | 320 | 96 |
| 3 | 1280 | 83 |

Varying concentrations of Human PYY were added to three different human plasma samples and the PYY content was determined by RIA. Mean of the observed levels from duplicate determinations in one assay are shown. Percent recovery was calculated as the observed over expected multiplied by 100.

### F. Linearity

Effect of Plasma Dilution

| Sample No. | Volume sampled | Observed pg/mL | Expected pg/mL | % Expected |
|---|---|---|---|---|
| 1 | 100µl | 161 | 161 | 100 |
| | 75µl | 162 | | 101 |
| | 50µl | 170 | | 105 |
| | 25µl | 180 | | 112 |
| 2 | 100µl | 156 | 156 | 100 |
| | 75µl | 167 | | 107 |
| | 50µl | 169 | | 108 |
| | 25µl | 179 | | 115 |
| 3 | 100µl | 199 | 199 | 100 |
| | 75µl | 220 | | 111 |
| | 50µl | 217 | | 109 |
| | 25µl | 246 | | 124 |
| 4 | 100µl | 124 | 124 | 100 |
| | 75µl | 125 | | 101 |
| | 50µl | 133 | | 107 |
| | 25µl | 155 | | 125 |

Aliquots of pooled Human Plasma containing varying concentrations of PYY were analyzed in the volumes indicated. Dilution factors of 1, 1.33, 2, and 4 representing 100 µL, 75 µL, 50 µL, and 25 µL respectively, were applied in calculating observed concentrations.

## XII. QUALITY CONTROLS

Good laboratory practice requires that quality control specimens be run with each standard curve to check the assay performance. Two levels of controls are provided for this purpose. These and any other control materials should be assayed repeatedly to establish mean values and acceptable ranges. Each individual laboratory is responsible for defining their system for quality control decisions and is also responsible for making this system a written part of their laboratory manual. The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD Millipore website emdmillipore.com using the catalog number as the keyword.

Recommended batch analysis decision using two controls (Westgard Rules[4]):

1. When both controls are within ±2 SD.
   Decision: Approve batch and release analyte results.
2. When one control is outside ±2 SD and the second control is within ±2 SD.
   Decision: Hold results, check with supervisor. If no obvious source of error is identified by the below mentioned check of systems, the supervisor may decide to release the results.

   Technician check of systems:
   1. Check for calculation errors
   2. Repeat standards and controls
   3. Check reagent solutions
   4. Check instrument

## XIII. REFERENCES


1. Morgan, C.R. and Lazarow, A. Immunoassay of Insulin: Two antibody system. Plasma insulin levels in normal, Subdiabetic, and diabetic rats. Diabetes 12:115-126, 1963.
2. Thorell, J.I. *Scand. J. Clin. Lab. Invest*. 31:187, 1973.
3. Feldman, H. and Rodbard, D. "Mathematical Theory of Radioimmunoassay", in: W.D. Odell and Doughaday, W.H. (Ed.), Principles of Competitive Protein-Binding Assays. Philadelphia: J.B. Leppincott Company; pp 158-203, 1971.
4. Westgard, J.O., et. al. A multi-rule Shewhart chart for quality control in clinical chemistry. *Clin. Chem*. 27:493-501, 1981.


## XIV. REPLACEMENT REAGENTS

| Reagent | Cat # |
|---|---|
| $^{125}$I-Human PYY (<1.5 µCi, 56 kBq) | 9066-HK |
| Guinea Pig Carrier (2 mL) | GPC-HK |
| Human PYY Standard | 8066-K |
| Human PYY (Total) Antibody (13 mL) | 1066-HK |
| Precipitating Reagent (130 mL) | PR-UVHK |
| Human PYY Quality Control 1&2 (1 mL each) | 6066-K |
| Assay Buffer (25 mL) | AB-66HK |

## XV. ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

### Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

### Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds.