Rat/Mouse PYY

125 Tubes

Cat. # RMPYY-68HK

RAT / MOUSE PYY RIA KIT

125 TUBES (Cat. # RMPYY-68HK)

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EMD Millipore Corporation • 6 Research Park Drive • St. Charles, Missouri 63304 USA
Phone: 636-441-8400 • Toll Free U.S.: 866-441-8400 • Fax: 636-441-8050
techserv.dd@merckgroup.com • www.millipore.com/bmia

RAT / MOUSE PYY RIA KIT
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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 when infused into subjects has been shown to reduce food intake in normal weight and obese individuals. Its 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[1,2]. In circulation, PYY exists in at least two molecular forms: (1-36) and (3-36)[3].

EMD Millipore's Rat/Mouse PYY Radioimmunoassay (RIA) Kit utilizes an antibody, which recognizes both 1-36 and 3-36 form of Rat and Mouse PYY. Assay sensitivity of 15.6 pg/mL can easily be achieved when using a 20 µl serum or plasma sample in a two-day, disequilibrium assay. ***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 Rat/Mouse PYY assay utilizes ^{125}I-labeled Rat PYY and a Rat PYY antiserum to determine the level of 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

Buffer containing BSA and 0.08% sodium azide
Quantity: 20 mL/vial, 2 bottles
Preparation: Ready to use

B. Rat PYY Antibody

Rabbit anti-Rat PYY Serum in Assay Buffer
Quantity: 13 mL/vial
Preparation: Ready to use

C. ^{125}I-Rat PYY

^{125}I-Rat PYY Label (<1.5 µCi, <56 kBq)
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. Rat PYY Standard

Synthetic lyophilized Rat PYY standard in Assay Buffer
Lyophilized for stability.
Quantity: 1 mL/vial upon hydration
Preparation: Contents Lyophilized. Hydrate with 1 mL distilled or deionized water. The actual concentration of rat PYY present in the vial will be lot-dependent. Please refer to the analysis sheet for exact rat PYY concentration present in a specific lot.

E. Rat PYY Quality Controls 1 & 2

Recombinant lyophilized Rat 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. Matrix Solution (For Serum Samples Only)

Treated animal serum
Quantity: 0.5 mL/vial
Preparation: Ready to use

G. Rabbit Carrier

Diluted rabbit serum
Quantity: 2 mL/vial
Preparation: Ready to use

III. REAGENTS SUPPLIED (continued)

H. Precipitating Reagent

Goat anti-Rabbit 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

Refrigerate all reagents between 2 and 8°C for short-term storage. For prolonged storage (>2 weeks), freeze at ≤ -20°C. 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. Store remaining hydrated Standard, Quality Controls and Tracer at -20°C.

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.

V. REAGENT PRECAUTIONS (continued)

A. Radioactive Materials (continued)

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 has been added to all reagents as a preservative at a concentration of 0.08%. Although it is at a minimum concentration, Sodium Azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

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. 20 µL and 100 µL pipettes 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)
10. DPP-IV inhibitor (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 and the addition of 10 µL of DPP-IV inhibitor per mL of blood.

1. A minimum of 20 µL per assay tube of serum or plasma should be used. Tissue culture and other media may also be used but the volume required for the assay will vary on incubation conditions, cell type, and cell concentration.
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. Rat PYY Standard Preparation

Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the PYY Standard with **1 mL** distilled or deionized water into the glass vial to give the concentration described in the analysis sheet. Invert and mix gently, let sit for five minutes or until completely dissolved then mix well.

Label five glass tubes 1, 2, 3, 4, and 5. Add 0.5 mL Assay Buffer to each of the five 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.

Note: 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)	1 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

B. PYY Quality Control 1 and 2 Preparation

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.

IX. ASSAY PROCEDURE (continued)

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 (B_0) tubes (5-6). Pipette 100 µL of Assay Buffer to the Standard tubes (7-18) and Control tubes (19-22). Pipette 200 µL of Assay Buffer in sample tubes 23 through the end of the assay.
2. **For Serum Samples**: Pipette 20 µL of Matrix Solution to the Non-Specific Binding (NSB) tubes (3-4), Reference (B_0) tubes (5-6), Standard tubes (7-18) and Quality Control tubes (19-22).

   For Plasma Samples: Pipette 20 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4), Reference (B_0) tubes (5-6), Standard tubes (7-18) and Quality Control tubes (19-22).
3. Pipette 100 µL of each Standard (tubes 7-18) and Quality Controls (tubes 19-22).
4. Pipette 20 µL of each sample in duplicate.
5. Pipette 100 µL of Rat PYY Antibody to all tubes except Total Count tubes (1-2) and NSB tubes (3-4).
6. Vortex, cover, and incubate overnight (20-24 hours) at 4ºC.

Day Two

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

Day Three

9. Add 10 µL of Rabbit Carrier to all tubes except Total Count the tubes (1-2).
10. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes except Total Count tubes (1-2).
11. Vortex and incubate 20 minutes at 4°C.
12. 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:

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

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

    rpm = revolutions per minute
13. 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.

Assay Procedure Flow Chart

Day 1						Day 2		Day 3		
Set-up	Step 1	Step 2	Step 3&4	Step 5	Step 6	Step 7	Step 8	Step 9	Step 10	Steps -11
Tube Number	Add Assay Buffer	Add Matrix Solution(serum samples) or Assay Buffer (plasma samples)	Add Standard/QC Sample	Add PYY (3-36) Antibody	**Vortex, Cover, and Incubate 20-24 hrs at 4°C**	Add I-125 Rat PYY Tracer	**Vortex, Cover and Incubate 22-24 hrs at 4°C**	Add Rabbit 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	20 µl	--	--		100 µl		10 µl	1.0 mL	
5,6	200 µl	20 µl	--	100 µl		100 µl		10 µl	1.0 mL	
7,8	100 µl	20 µl	100 µl of tube 5	100 µl		100 µl		10 µl	1.0 mL	
9,10	100 µl	20 µl	100 µl of tube 4	100 µl		100 µl		10 µl	1.0 mL	
11,12	100 µl	20 µl	100 µl of tube 3	100 µl		100 µl		10 µl	1.0 mL	
13,14	100 µl	20 µl	100 µl of tube 2	100 µl		100 µl		10 µl	1.0 mL	
15,16	100 µl	20 µl	100 µl of tube 1	100 µl		100 µl		10 µl	1.0 mL	
17,18	100 µl	20 µl	100 µl of reconstituted standard	100 µl		100 µl		10 µl	1.0 mL	
19,20	100 µl	20 µl	100 µl of QC 1	100 µl		100 µl		10 µl	1.0 mL	
21,22	100 µl	20 µl	100 µl of QC 2	100 µl		100 µl		10 µl	1.0 mL	
23,n	200 µl	--	20 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, B_0), (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.
8. Multiply all the sample values by a factor of 5, since only 20 µL sample is used against 100 µL standard.

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 15.6 pg/mL when using a 100 µL sample size. The lowest level of PYY that can be detected by this assay when using a 20 µL sample size is 78.1 pg/mL.

B. Performance

The following parameters of assay performance are expressed as Mean ± Standard Deviation from 7 different assays

ED_{20} = 190.2 ± 16.3 pg/mL

ED_{50} = 90.7 ± 10.9 pg/mL

ED_{80} = 41.9 ± 7.2 pg/mL

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.

Cross reactivity of different analytes in Rat PYY RIA

Analyte	% Cross-Reactivity
Rat, Mouse, Canine, Porcine PYY 3-36	100.0
Rat, Mouse, Canine, Porcine PYY 1-36	100.0
Human PYY 3-36	70.0
Human PYY 1-36	70.0
Rat NPY (1000 pg/mL)	ND
GLP-1 (100 pM)	ND
Glucagon (pg/mL)	ND
Rat/Mouse GIP (2000 pg/mL)	ND
Rat PP (10,000 pg/mL)	ND
Rat Ghrelin (10,000 pg/mL)	ND

ND - Not detectable up to the concentration shown in parenthesis.

XI. ASSAY CHARACTERISTICS (continued)

D. Precision

Intra- and Inter-Assay Variation:

Sample no.	Mean pg/mL	Intra-Assay %CV	Inter-Assay %CV
1	59.1	3.9	7.6
2	131.5	3.2	9.4

Intra- and inter-assay variations were performed on two samples containing low and high concentrations of Rat PYY. Data (mean and %CV) shown are from one assay with ten duplicate determinations of each sample for intra-assay precision. For inter-assay precision, data are generated using seven separate assays run for the two high and low samples in duplicate.

E. Spike and Recovery

Rat PYY (3-36) pg/mL	% Expected
40	97.5 ± 1.5
80	97.0 ± 6.4
160	101.3 ± 5.0

Four different rat plasma samples were spiked with different amounts of exogenous rat PYY (3-36). These spiked plasma samples were assayed by Rat PYY RIA. Expected values are the basal levels plus the spiked amount (40, 80 and 160pg/mL) of PYY. The % Expected is observed value divided by expected value X 100 (Mean ± SD).

XI. ASSAY CHARACTERISTICS (continued)

F. Linearity and Dilution

Sample No.	Basal Rat Plasma % Expected	Spiked Rat Plasma % Expected	Basal Mouse Plasma % Expected	Spiked Mouse Plasma % Expected
12.5µl	125.8 ± 22.7	96.6 ± 28.5	83.6 ± 8.1	83.9 ± 3.5
25µl	114.9 ± 7.0	92.1 ± 12.8	81.5 ± 2.4	92.5 ± 8.3
50µl	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Four different basal (non-spiked) and spiked (with exogenous Rat PYY 3-36) rat and mouse plasma samples at 12.5, 25 and 50 µL were assayed by Rat PYY RIA using 50 µL matrix solution after adding the remainder of 50 µl sample volume with matrix solution. % Expected values (mean ± SD) are 1/4, 1/2 and 1/1 of the 50 µL sample value.

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 www.Millipore.com/bmia.

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. Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatei, M.A., Cone, R.D., Bloom, S.R. Gut Hormone PYY physiologically inhibits food intake. *Nature*. 418:650-4, 2002, Aug.

2. Batterham, R.L., Cohen, M.A., Ellis, S.M., Roux, C.W., Withers, D.J., Frost, G.S., Ghatei, M.A., Bloom, S.R. Inhibition of food intake in obese subjects by Peptide YY (3-36). *N. Engl. J. Med.* 349(10):941-8, 2003, Sept.

3. Grandt, D., Schimiczek, M., Beglinger, C., Layer, P., Goebell, H., Eysselein, V.E., Reeve, J.R. Two molecular forms of Peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. *Regulatory Peptides.* 51(1994):151-9, 1994.

4. Westgard, J.O., et. al. A multi-rule Shewhart chart for quality control in clnical chemistry. *Clin. Chem*. 27:493-501, 1981.

XIV. REPLACEMENT REAGENTS

Reagent	Cat #
^{125}I-Rat PYY (<1.5 uCi, 56 kBq)	9068-HK
Rabbit Carrier	RC-HK
Rat PYY Standard	8068-K
Rat PYY Antibody (13 mL)	1068-HK
Precipitating Reagent (130 mL)	PR-81HK
Rat PYY Quality Control 1 & 2 (1 mL each)	6068-K
Assay Buffer (20 mL)	AB-66HK
Matrix solution	MTX-PYY

XV. ORDERING INFORMATION

To place an order:

For USA Customers:

To assure the clarity of your custom panel order, please FAX the following information to our customer service department:

- Your name, telephone and/or fax number
- Customer account number
- Shipping and billing address
- Purchase order number
- Catalog number and description of product
- Quantity of kits

NOTE: Appropriate license from NRC (or equivalent) must be on file at EMD Millipore before radioactive orders can be shipped.

TELEPHONE ORDERS:

Toll Free US: (800) MILLIPORE

FAX ORDERS: (636) 441-8050

MAIL ORDERS: EMD Millipore Corporation
6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers in placing an order or obtaining additional information about MILLIPLEX® MAP products, please contact your multiplex specialist or sales representative or email our European Customer Service at customerserviceEU@Millipore.com.

Conditions of Sale

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

Material Safety Data Sheets (MSDS)

Material safety data sheets for EMD Millipore products may be ordered by fax or phone. See Section A above for details on ordering.