Rat Pancreatic Polypeptide RIA Kit
125 Tubes (Cat. # RPP- 41HK)

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I. INTENDED USE

Rat pancreatic polypeptide (PP) is a 36 amino acid polypeptide (Molecular Weight: 4399) which is predominantly secreted by the pancreatic Islet of Langerhans (1). Its physiological role is not precisely understood. However, it has been shown to affect the secretion of pancreatic enzymes, water and electrolytes (1). It also influences gastric emptying and gut motility. Food ingestion, prolonged fasting, diabetes and exercise may influence its secretion (1). Pancreatic Polypeptide hormone secretion could be increased in certain pancreatic tumors and may be decreased in pancreatitis (2,3). Measurements of circulating levels of pancreatic polypeptide in health and disease could be important in understanding the etiology and/or patho-physiology of diabetes, obesity and gastro-intestinal disorders.

Millipore's Rat Pancreatic Polypeptide Radioimmunoassay (RIA) Kit utilizes an antibody that recognizes rat PP. Assay sensitivity of 0.15ng/mL can be achieved when using 50 µl rat serum or plasma sample in a two-day, disequilibrium assay. ***This kit is for research purposes only.***

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 Millipore Rat Pancreatic Polypeptide assay utilizes ^{125}I-labeled Rat Pancreatic Polypeptide and a Rat Pancreatic Polypeptide antiserum to determine the level of Pancreatic Polypeptide 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/bottle, 2 bottles
Preparation: Ready to use

B. Rat Pancreatic Polypeptide Antibody

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

C. ^{125}I- Rat Pancreatic Polypeptide

^{125}I-Rat Pancreatic Polypeptide 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 entire 13.5 mL of Assay Buffer. Allow to set at room temperature for 30 minutes, with occasional gentle mixing.

D. Rat Pancreatic Polypeptide Standard

Recombinant lyophilized Rat Pancreatic Polypeptide (3-36) standard in Assay Buffer
Lyophilized for stability.
Quantity: 1 mL/vial upon hydration (10 ng/mL)
Preparation: Contents lyophilized. Hydrate with 1 mL distilled or deionized water. The actual concentration of rat PP present in the vial will be lot-dependent. Please refer to the analysis sheet for exact rat PP concentration present in the specific lot.

E. Rat Pancreatic Polypeptide Quality Controls 1 & 2

Recombinant lyophilized Rat Pancreatic Polypeptide 3-36 in Assay Buffer.
Lyophilized for stability.
Quantity: 1 mL/vial upon hydration.
Preparation: Contents Lyophilized. Hydrate with 1 mL distilled or de-ionized water.

F. Matrix Solution

Treated animal serum
Quantity: 1.5 mL/vial
Preparation: Ready to use

III. REAGENTS SUPPLIED (continued)

G. Goat Carrier

Normal Goat Serum
Quantity: 2 mL/vial
Preparation: Ready to use

H. Precipitating Reagent

Anti-Goat IgG Serum, 3% PEG and 0.05% Triton X-100 in 0.05M Phosphosaline, 0.025M EDTA, 0.08% Sodium Azide
Quantity: 260 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 all the reagents 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.

V. REAGENT PRECAUTIONS (continued)

A. Radioactive Materials (continued)

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 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. 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

VII. SPECIMEN COLLECTION AND STORAGE

1. A minimum of 50 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 heparin may 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 Pancreatic Polypeptide Standard Preparation

Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Rat Pancreatic Polypeptide Standard with **1 mL** distilled or de-ionized water into the glass vial to give the highest standard concentration. Invert and mix gently, let sit for five minutes or until completely dissolved then mix well.

Label six glass tubes 1, 2, 3, 4, 5 and 6. Add 0.5 mL Assay Buffer to each of the six 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 and mix well.

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℃. Avoid multiple freeze/thaw cycles.

VIII. ASSAY PROCEDURE (continued)

A. Rat Pancreatic Polypeptide Standard Preparation (continued)

	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 ng/mL	Volume of Assay Buffer to Add	Volume of Standard to Add
1	X/2	0.5 mL	0.5 mL of Original 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

B. Rat Pancreatic Polypeptide Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Using a pipette, reconstitute each of the Rat Pancreatic Polypeptide 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.

VIII. ASSAY PROCEDURE (continued)

C. Assay Set Up

Day One

1. Pipette 250 µL of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4). Pipette 150 µL of Assay Buffer in the Reference (B_0) tubes (5-6). Pipette 100 µl Assay Buffer to the Standard tubes (7-20) and Control tubes (21-24). Pipette 150 µL of Assay Buffer in sample tubes 25 through the end of the assay.
2. Pipette 50 µL of Matrix Solution to the Non-Specific Binding (NSB) tubes (3-4), Reference (B_0) tubes (5-6) and Standard tubes (7-20) and Quality Control tubes (21-24).
3. Pipette 50 µL of each Standard (tubes 7-20) and Quality Controls (tubes 21-24).
4. Pipette 50 µL of each sample in duplicate.
5. Pipette 100 µL of Rat Pancreatic Polypeptide 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 Pancreatic Polypeptide tracer with 13.5 mL of Assay Buffer and gently mix. Pipette 100 µl of ^{125}I-Rat Pancreatic Polypeptide to all tubes.
8. Vortex, cover and incubate overnight (22-24 hours) at 4ºC.

Day Three

9. Add 10 µL of Goat Carrier to all tubes except Total Count tubes (1-2).
10. Add 1.0 mL of cold (4°C) Precipitating Reagent to all tubes except Total Count the 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 x 10-5) ® (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	Steps 3&4	Step 5	Step 6	Step 7	Step 8	Step 9	Step 10	Steps 11-13
Tube Number	Add Assay Buffer	Add Matrix Solution	Add Standard/QC Sample	Add Rat PP Antibody	**Vortex, Cover, and Incubate 20-24 hrs at 4°C**	Add I^{-125} Rat PP Tracer	**Vortex, Cover and Incubate 22-24 hrs at 4°C**	Add Goat 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	250 µL	50 µL	--	--		100 µL		10 µL	1.0 mL	
5,6	150 µL	50 µL	--	100 µL		100 µL		10 µL	1.0 mL	
7,8	100 µL	50 µL	50 µL of tube 6	100 µL		100 µL		10 µL	1.0 mL	
9,10	100 µL	50 µL	50 µL of tube 5	100 µL		100 µL		10 µL	1.0 mL	
11,12	100 µL	50 µL	50 µL of tube 4	100 µL		100 µL		10 µL	1.0 mL	
13,14	100 µL	50 µL	50 µL of tube 3	100 µL		100 µL		10 µL	1.0 mL	
15,16	100 µL	50 µL	50 µL of tube 2	100 µL		100 µL		10 µL	1.0 mL	
17,18	100 µL	50 µL	50 µL of tube 1	100 µL		100 µL		10 µL	1.0 mL	
19,20	100 µL	50 µL	50 µL of Original Standard	100 µL		100 µL		10 µL	1.0 mL	
21,22	100 µL	50 µL	50 µL of QC 1	100 µL		100 µL		10 µL	1.0 mL	
23,24	100 µL	50 µL	50 µL of QC 2	100 µL		100 µL		10 µL	1.0 mL	
25,n	150 µL	--	50 µL of unknown	100 µL		100 µL		10 µL	1.0 mL	

IX. CALCULATIONS

A. Explanation

The calculations for Rat Pancreatic Polypeptide 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 30-50%.
4. Calculate the percentage of total binding (%B/B_0) for each standard and sample

   %B/B_0 = (Sample or Standard/Total Binding) X 100
5. Plot the % B/B_0 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 Rat Pancreatic Polypeptide in the unknown samples and controls by interpolation of the reference curve.

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 Rat Pancreatic Polypeptide that can be detected by this assay is 0.156 ng/mL when using a 50 µL sample size.

B. Performance

The following parameters of assay performance are expressed as Mean ± Standard Deviation from 3 different assays

ED_{20} = 3.17 ± 0.53 ng/mL
ED_{50} = 0.91 ± 0.12 ng/mL
ED_{80} = 0.27 ± 0.04 ng/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 Pancreatic Polypeptide RIA

Analyte	% Cross-Reactivity
Rat Pancreatic Polypeptide	100.0
Mouse Pancreatic Polypeptide	31%
Human NPY (50 ng/mL)	ND
Rat NPY (50 ng/mL)	ND
GLP-1 (500 pM)	ND
Glucagon (400 pg/mL)	ND
Rat/Mouse GIP (2000 pg/mL)	ND
Rat PYY (500 pg/mL)	ND
Rat Ghrelin 10,000 pg/mL)	ND
Human Insulin (200 µU/mL)	ND
Rat Leptin (50 ng/mL)	ND
Human Pancreatic Polypeptide (10 ng/mL)	0.4
Human Adiponectin (200 ng/mL)	0.1

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	0.46	7.6	5.7
2	1.96	7.9	9.9

Intra- and Inter-assay variations were performed on two samples containing low and high concentrations of Rat Pancreatic Polypeptide. 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 six separate assays run for the two high and low samples in duplicate.

F. Spike and Recovery

Sample Type	Rat Serum (n=3) % Expected	Rat Plasma (n=3) % Expected
0.312 ng/mL	92.2 ± 7.0	98.6 ± 26.3
0.625 ng/mL	95.9 ± 14.5	92.1 ± 25.6
1.25 ng/mL	93.4 ± 8.4	93.6 ± 16.6
2.5 ng/mL	87.1 ± 11.7	88.4 ± 12.9

Rat serum and plasma samples were spiked with different amounts of exogenous rat Pancreatic Polypeptide. These spiked serum and plasma samples were assayed by Rat Pancreatic Polypeptide RIA. Expected values are the basal levels plus the spiked amount (0.312, 0.625, 1.25 and 2.5 ng/mL) of Pancreatic Polypeptide. The % Expected is observed value divided by expected value X 100 (Mean ± SD).

XI. ASSAY CHARACTERISTICS (continued)

F. Linearity and Dilution

Sample Type Dilution Factor	Rat Serum (n=2) % Expected	Rat Plasma (n=2) % Expected
1/4	77.4 ± 4.1	70.7 ± 16.8
1/2	92.1 ± 0.4	87.7 ± 23.6
1/1	100.0 ± 0.0	100.0 ± 0.0

Rat serum and plasma samples were spiked with 2.5 ng/mL of exogenous rat Pancreatic Polypeptide. Then these spiked samples were diluted ½ and ¼ with matrix and assayed by Rat Pancreatic Polypeptide RIA. % 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 Millipore website www.millipore.com.

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

1. When both controls are within ±2 SD.
   Decision: Approve pancreatic polypeptide 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. Henderson RA and Rinker AD, "Gastric, Pancreatic and Intestinal Function," Burtis CA and Ashwood ER, eds, *Tietz Textbook of Clinical Chemistry*, 3rd ed, Philadelphia, PA: WB Saunders Co, 1999, 1271-1327.
2. Pasieka JL and Hershfield N, "Pancreatic Polypeptide Hyperplasia Causing Watery Diarrhea Syndrome: A Case Report," *Can J Surg*, 1999, 42(1):55-8 (review).
3. Vinik AI, Strodel WE, Eckhauser FE, et al, "Somatostatinomas, Pancreatic Polypeptideomas, Neurotensinomas," *Semin Oncol*, 1987, 14(3):263-81.
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-Rat Pancreatic Polypeptide (<1.5 µCi, 56 kBq)	9041-HK
Goat Carrier (2 mL)	GTC-HK
Rat Pancreatic Polypeptide Standard	8041-K
Rat Pancreatic Polypeptide Antibody (13 mL)	1041-HK
Precipitating Reagent (260 mL)	PR-15
Rat Pancreatic Polypeptide Quality Control 1&2 (1 mL each)	6041-K
Assay Buffer (20 mL)	AB-66HK
Matrix solution (1.5 mL)	MTX-RS

XV. ORDERING INFORMATION

A. To place an order:

For USA Customers:

Please provide the following information to our customer service department to expedite your telephone, fax or mail order:

1. Your name, telephone and/or fax number
2. Customer account number
3. Shipping and billing address
4. Purchase order number
5. Catalog number and description of product
6. Quantity and product size

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

TELEPHONE ORDERS:

Toll Free US (866) 441-8400
(636) 441-8400

FAX ORDERS: (636) 441-8050

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

For International Customers:

To best serve our international customers, it is Millipore's policy to sell our products through a network of distributors. To place an order or to obtain additional information about Millipore products, please contact your local distributor.

B. Conditions of Sale

All products are for research or manufacturing use only. They are not intended for use in clinical diagnosis or for administration to humans or animals. All products are intended for *in vitro* use only.

C. Material Safety Data Sheets (MSDS)

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