

**HUMAN PYY (3-36) SPECIFIC RIA KIT
125 TUBES (Cat. # PYY-67HK)**

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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 (3-36) infusion also reduced the plasma levels of the hunger-promoting hormone ghrelin. PYY (3-36) levels have been shown to drop pre-meal and then increase post prandially^{1,2}. In circulation, PYY (3-36) exists in at least two molecular forms: (1-36) and (3-36)³.

Linco's PYY (3-36) Radioimmunoassay (RIA) Kit utilizes an antibody, which recognizes only 3-36 form of Human PYY (3-36). Sensitivity of 20 pg/ml can easily be achieved when using a 100µl 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 Linco Research PYY (3-36) assay utilizes ¹²⁵I-labeled PYY and a PYY (3-36) antiserum to determine the level of PYY (3-36) 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

Preparation: Ready to use

B. PYY (3-36) Antibody

Guinea Pig anti-PYY (3-36) Serum in Assay Buffer

Quantity: 13 ml/vial

Preparation: Ready to use

C. ¹²⁵I-PYY

¹²⁵I-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 entire contents of Label Hydrating Buffer. Allow to set at room temperature for 30 minutes, with occasional gentle mixing.

D. Human PYY Label Hydrating Buffer

Assay Buffer containing normal guinea pig serum as carrier.

Used to hydrate ¹²⁵I-PYY.

Quantity: 13.5 ml/vial

Preparation: Ready to use

E. Human PYY Standard

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.

F. Human PYY 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.

III. REAGENTS SUPPLIED (continued)

G. Matrix Solution

Treated human serum

Quantity: 2.5 ml/vial

Preparation: Ready to use; chill to 4°C.

H. 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

Refrigerate all reagents between 2 and 8°C for short-term storage. For prolonged storage (>2 weeks), freeze at $\leq -20^{\circ}\text{C}$. Avoid multiple (>5) freeze/thaw cycles. Refer to date on bottle for expiration when stored at $\leq -20^{\circ}\text{C}$. 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)

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.

C. Matrix Solution:

Matrix solution is treated human serum. Although negative for HIV and Hepatitis virus, all precautions should be taken to avoid any possible contamination. Dispose of all material coming in contact with this solution as BIO-HAZARD.

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 (3-36). 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 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 $\leq -20^{\circ}\text{C}$ 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 the concentration described in the analysis sheet. 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 $\leq -20^{\circ}\text{C}$. 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

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 $\leq -20^{\circ}\text{C}$. Avoid multiple freeze/thaw cycles.

VIII. ASSAY PROCEDURE (continued)

Day One

1. Pipette 200 µl of Assay Buffer to the Non-Specific Binding (NSB) tubes (3-4). Pipette 100 µl of Assay Buffer in the Reference (Bo) tubes (5-6) and sample tubes 25 through the end of the assay. **Do not add buffer to standard and QC tubes.**
2. Pipette 100 µl of Matrix Solution to the Non-Specific Binding (NSB) tubes (3-4), Reference (Bo) tubes (5-6) and Standard tubes (7-20) and Quality Control tubes (21-24).
3. Pipette 100 µl of each Standard (tubes 7-20) and Quality Controls (tubes 21-24).
4. Pipette 100 µl of each sample in duplicate. (NOTE: Smaller volumes of sample may be used when PYY (3-36) 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.
5. Pipette 100 µl of PYY (3-36) 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 4°C.

Day Two

7. Hydrate the ¹²⁵I-PYY tracer with 13.5 ml of Label Hydrating Buffer and gently mix. Pipette 100 µl of ¹²⁵I-PYY to all tubes.
8. Vortex, cover and incubate overnight (22-24 hours) at 4°C.

Day Three

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:
$$xg = (1.12 \times 10^{-5}) \textcircled{R} (\text{rpm})^2$$

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.

Assay Procedure Flow Chart

Day One						Day Two		Day Three	
Set-up	Step 1	Step 2	Step 3&4	Step 5	Step 6	Step 7	Step 8	Step 9	Steps 9-11
Tube Number	Add Assay Buffer	Add Matrix Solution	Add Standard/QC Sample	Add PYY (3-36) 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 Precipitating Reagent	Incubate 20 min. at 4°C, Centrifuge at 4°C for 20 min Decant and Count
1,2	-	-	-	-		100 µl		-	
3,4	200 µl	100 µl	-	-		100 µl		1.0 ml	
5,6	100 µl	100 µl	-	100 µl		100 µl		1.0 ml	
7,8	-	100 µl	100 µl of tube 6	100 µl		100 µl		1.0 ml	
9,10	-	100 µl	100 µl of tube 5	100 µl		100 µl		1.0 ml	
11,12	-	100 µl	100 µl of tube 4	100 µl		100 µl		1.0 ml	
13,14	-	100 µl	100 µl of tube 3	100 µl		100 µl		1.0 ml	
15,16	-	100 µl	100 µl of tube 2	100 µl		100 µl		1.0 ml	
17,18	-	100 µl	100 µl of tube 1	100 µl		100 µl		1.0 ml	
19,20	-	100 µl	100 µl of reconstituted standard	100 µl		100 µl		1.0 ml	
21,22	-	100 µl	100 µl of QC 1	100 µl		100 µl		1.0 ml	
23,24	-	100 µl	100 µl of QC 2	100 µl		100 µl		1.0 ml	
25,n	100 µl	-	100 µl of unknown	100 µl		100 µl		1.0 ml	

IX. CALCULATIONS

A. Explanation

The calculations for PYY (3-36) 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.

$$(\text{Total Binding Counts}/\text{Total Counts}) \times 100$$

This should be 35-50%.

4. Calculate the percentage of total binding (%B/Bo) for each standard and sample

$$\%B/Bo = (\text{Sample or Standard}/\text{Total Binding}) \times 100$$

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 (3-36) in the unknown samples and controls by interpolation of the reference curve.

[NOTE: When sample volumes assayed differ from 100 μ l, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 50 μ l 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 (3-36) that can be detected by this assay is 20 pg/ml when using a 100µl sample size.

B. Performance

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

$$ED_{80} = 93 \pm 3$$

$$ED_{50} = 230 \pm 15$$

$$ED_{20} = 558 \pm 44$$

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 (3-36) RIA Crossreactivity

PYY (3-36) human 100%

PYY (1-36) human Not detectable up to 1000 pg/ml

ND-Not detectable up to 1000 pg/ml

D. Precision

Within and Between Assay Variation

Sample no.	Mean pg/ml	Within %CV	Between %CV
1	84	11.0	15.0
2	217	6.4	7.0

Within and between assay variations were performed on two samples containing low and high concentrations of Human PYY (3-36). 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 eight separate assays run for the two high and low samples in duplicate.

XI. ASSAY CHARACTERISTICS (continued)

F. Spike and Recovery

Sample No.	PYY (3-36) pg/ml added	Observed pg/ml	Expected pg/ml	% Expected
1	0	71.0	71.0	100.0
	80	138.1	151.0	91.4
	160	215.9	231.0	93.5
	320	349.6	391.0	89.4
	640	670.2	711.0	94.3
2	0	61.2	61.2	100.0
	80	126.2	141.2	89.3
	160	193.7	221.2	87.5
	320	354.1	381.2	92.9
	640	632.1	701.2	90.1
3	0	62.5	62.5	100.0
	80	126.0	142.5	88.4
	160	189.3	222.5	85.1
	320	354.8	382.5	92.7
	640	644.1	702.5	91.7
4	0	62.2	62.2	100.0
	80	139.8	142.2	98.3
	160	209.2	222.2	94.1
	320	399.1	382.2	104.4
	640	905.9	702.2	129.0

Four different plasma samples were spiked with different amounts of exogenous PYY (3-36). These spiked plasma samples were assayed by PYY (3-36) RIA. Expected values are the basal levels plus the spiked amount (80, 160, 320 and 640 pg/ml) of PYY (3-36). Then % Expected is observed value divided by expected value.

XI. ASSAY CHARACTERISTICS (continued)

G. Linearity

Effect of Plasma Dilution

Sample No.	Volume sampled	Observed pg/ml	Expected pg/ml	% Expected
1	50µl	39.8	42.1	94.6
	75µl	85.0	63.1	134.8
	100µl	84.1	84.1	100.0
2	50µl	27.5	30.1	91.3
	75µl	37.3	45.2	82.6
	100µl	60.2	60.2	100.0
3	50µl	36.0	30.3	118.6
	75µl	39.2	45.5	86.1
	100µl	60.7	60.7	100.0
4	50µl	34.0	35.1	96.8
	75µl	53.2	52.7	101.0
	100µl	70.2	70.2	100.0

Four different plasma samples at 50, 75 and 100 ul were assayed by PYY (3-36) RIA after adding the remainder of 100 ul sample volume with matrix solution. Expected values are $\frac{1}{2}$, $\frac{3}{4}$ and $\frac{1}{1}$ of the 100 ul sample value.

Effect of Exogenously Spiked Plasma Dilution

Sample No.	Volume sampled	Observed pg/ml	Expected pg/ml	% Expected
1	25µl	175.9	151.2	116.3
	50µl	311.7	302.4	103.1
	75µl	498.3	453.6	109.8
	100µl	604.8	604.8	100.0
2	25µl	167.2	145.3	115.1
	50µl	317.6	290.7	109.3
	75µl	436.5	436.0	100.1
	100µl	581.3	581.3	100.0
3	25µl	167.7	207.6	80.8
	50µl	366.8	415.3	88.3
	75µl	542.2	622.9	87.0
	100µl	830.6	830.6	100.0

Three different plasma samples were first spiked with exogenous PYY (3-36). These spiked plasma samples at 25, 50, 75 and 100 ul volumes were assayed by PYY (3-36) RIA after adding the remainder of 100 ul sample volume with matrix solution. Expected values are $\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$ and $\frac{1}{1}$ of the 100 ul 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 Linco Research website www.lincoresearch.com.

Recommended batch analysis decision using two controls (Westgard Rules⁴):

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

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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 clinical chemistry. *Clin. Chem.* 27:493-501, 1981.

XIV. REPLACEMENT REAGENTS

Reagent	Cat #
¹²⁵ I-PYY (<1.5 uCi, 56 kBq)	9066-HK
Human PYY Label Hydrating Buffer (13.5 ml)	LHB-66HK
Human PYY Standard	8066-K
PYY (3-36) Antibody (13 ml)	1067-HK
Precipitating Reagent (130 ml)	PR-UVHK
Human PYY Quality Control 1&2 (1 ml each)	6066-K
Assay Buffer (20 ml)	AB-66HK
Matrix solution	HS0067

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 LINCO before radioactive orders can be shipped.

TELEPHONE ORDERS:

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

FAX ORDERS: (636) 441-8050

MAIL ORDERS: LINCO Research

6 Research Park Drive
St. Charles, Missouri 63304 U.S.A.

For International Customers:

To best serve our international customers, it is LINCO's policy to sell our products through a network of distributors. To place an order or to obtain additional information about LINCO 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 LINCO Research, Inc. products may be ordered by fax or phone. See Section A above for details on ordering.