Rat/Mouse Neuropeptide Y (NPY)

96 Well Plate Assay

Cat. # EZRMNPY-27K

RAT/MOUSE NEUROPEPTIDE Y (NPY) ELISA KIT
96-Well Plate (Cat. # EZRMNPY-27K)

RAT/MOUSE NPY ELISA KIT
96-Well Plate (Cat# EZRMNPY-27K)

I. INTENDED USE

This kit is used for the non-radioactive quantification of Neuropeptide Y (NPY) in rat/mouse plasma. One kit is sufficient to measure 38 unknown samples in duplicate. ***This kit is for research purpose only.***

II. PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based on: 1) capture of NPY molecules in the sample by anti-NPY IgG and immobilization of the resulting complex to the wells of a microtiter plate coated by a pre-titered amount of anchor antibodies, 2) and the simultaneous binding of a second biotinylated antibody to NPY, 3) wash away of unbound materials, followed by conjugation of horseradish peroxidase to the immobilized biotinylated antibodies, 4) wash away of free enzyme, and 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetra-methylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450nm, corrected from the absorbency at 590nm, after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured NPY in the unknown sample, the concentration of NPY can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of NPY.

III. REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

1. Microtiter Plate

Coated with pre-titered anchor antibodies.

Quantity: 1 Strip Plate

Preparation: Ready to use.

Note: Unused strips should be resealed in the foil pouch with the dessicant provided and stored at 2-8 °C.

2. Adhesive Plate Sealer

Quantity: 2 sheets

Preparation: Ready to use.

3. 10X HRP Wash Buffer Concentrate

10X concentrate of 50 mM Tris Buffered Saline containing Tween-20.

Quantity: 2 bottles containing 50ml each

Preparation: Dilute 1:10 with distilled or de-ionized water.

4. Rat/Mouse NPY Standard

Rat/Mouse NPY reference standard, 2 ng/ml, lyophilized

Quantity: 1 bottle, 2 ng/ml after reconstitution with appropriate amount of water.

Preparation: Hydrate thoroughly in distilled or de-ionized water immediately before use. Please refer to the analysis sheet for exact amount of water to be used since it will be lot dependent.

5. Quality Controls 1 and 2

One vial each, lyophilized, containing Rat/Mouse NPY at two different levels.

Quantity: 0.5 ml/vial upon hydration.

Preparation: Reconstitute each vial with 0.5 ml de-ionized water immediately before use.

6. Matrix Solution

Processed serum matrix containing 0.08% Sodium Azide

Quantity: 1 ml/vial

Preparation: Ready to use.

III. REAGENTS SUPPLIED

7. Assay Buffer

0.05 M phosphosaline, pH 7.4, containing 0.025 M EDTA, 0.08% sodium azide, and 1% BSA.

Quantity: 40 ml/vial

Preparation: Ready to use.

8. Rat/Mouse NPY Capture Antibody

Pre-titered capture antibody solution in buffer

Quantity: 3 ml/vial

Preparation: Mix 1:1 with Rat/Mouse NPY Detection Antibody before use according to § VIII. C.

9. Rat/Mouse NPY Detection Antibody

Pre-titered detection antibody solution in buffer

Quantity: 3 ml/vial

Preparation: Mix 1:1 with Rat/Mouse NPY Capture Antibody before use according to § VIII. C.

10. Enzyme Solution

Pre-titered streptavidin-horseradish peroxidase conjugate in buffer.

Quantity: 12 ml/vial

Preparation: Ready to use

11. Substrate

3, 3',5,5'-tetramethylbenzidine in buffer.

Quantity: 12 ml/vial

Preparation: Ready to use. Minimize the exposure to light.

12. Stop Solution

0.3 M HCl

Quantity: 12 ml/vial

Preparation: Ready to use.

[Caution: Corrosive Solution]

IV. STORAGE AND STABILITY

Prior to use, all components in the kit can be stored up to 2 weeks at 2-8°C. For longer storage (> 2 weeks), freeze diluted Wash Buffer, Assay Buffer, Matrix Solution and reconstituted Standards and Controls at ≤ –20°C. Minimize repeated freeze and thaw of the Standards and Quality Controls. Unused microtiter strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

V. REAGENT PRECAUTIONS

1. Sodium Azide

Sodium azide has been added to certain 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 pluming to form explosive metal azides. On disposal, flush with large volume of water to prevent azide build up.

2. Hydrochloric Acid

Hydrochloric acid is corrosive, can cause eye and skin burns. Harmful if swallowed. Causes respiratory and digestive tract burns. Avoid contact with skin and eye. Do not swallow or ingest.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and pipette tips: 10µl ~ 20 µl or 20µl ~ 100 µl
2. Multi-channel Pipettes and pipette tips: 5 ~ 50 µl and 50 ~ 300 µl
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. De-ionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm and 590nm
7. Orbital Microtiter Plate Shaker
8. Absorbent Paper or Cloth
9. Aprotinin is recommended for Sample Collection and Storage.
10. DPP-IV Inhibitor (we recommend Millipore Cat# DPP4-010) is required for Sample Collection and Storage.

VII. PLASMA SAMPLE COLLECTION AND STORAGE

1. **NPY (3~36) amide and smaller fragments have been reported to be present in the blood. For best results, we recommend NPY protection from proteolytic degradation by treating blood samples with Millipore DPP-IV Inhibitor (Cat# DPP4-010) and Aprotinin immediately after the blood is drawn.**

2. **Authentic NPY is present in platelets of many strains of rats and mice. Leakage of NPY from platelets after blood clotting makes assay results in serum highly variable depending on the rate of release. We therefore recommend using only platelet-poor plasma as sample.**

3. To prepare plasma sample, whole blood should be collected into a centrifuge tube containing enough K_3 EDTA to achieve a final concentration of 1.735 mg/ml. Immediately add enough DPP-IV Inhibitor and Aprotinin to a final concentration of 50 µM and 500 KIU/ml, respectively, and quickly centrifuge the blood at 2,000 to 3,000 x g for 15 minutes at 4 ± 2°C.

4. Transfer and aliquot plasma samples in separate tubes of small quantity. Date and identify each sample.

.

5. Use freshly prepared plasma or store samples at –20 ± 5°C for later use. Avoid multiple (> 5) freeze/thaw cycles.

6. If heparin is to be used as anti-coagulant, the effect on the assay outcome at the dose of heparin used should be pre-determined.

7. Avoid using samples with gross hemolysis or lipemia.

8. **Plasma NPY levels vary greatly amongst different strains of rats and mice. We have measured the basal level in 6 strains of mice (8 animals each from CD-1, Swiss Webster, C57BL/6, BALB/C, OB/OB and db/db) and 5 strains of rats (8 animals each from Sprague Dawley, Wistar Hannover, Fischer 344, Zucker Lean and Zucker Fatty). The plasma samples of two Zucker rat strains required 40-fold dilution with assay buffer and all other rat and mouse samples required a 10-fold dilution for this ELISA kit. Customer should determine the optimum dilution for their samples.**

   NOTE: When using diluted plasma samples, the matrix solution also must be diluted with assay buffer to the same degree as assayed samples (Refer to Section VIII. D). When working with other strains of rodents, the optimum dilution factor should be established first.

VIII. REAGENT PREPARATION

A. Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Rat/Mouse NPY Standard with the amount of distilled or deionized water specified in the data sheet supplied with this kit to give a final concentration of 2 ng/ml of NPY Standard. Invert and mix gently until completely in solution.

2. Label six tubes with the additional concentrations of standards to be prepared: 0.01 ng/ml, 0.04 ng/ml, 0.10 ng/ml, 0.25 ng/ml, 0.50 ng/ml and 1 ng/ml. Add Assay Buffer to each of the six tubes according to the volumes outlined in the chart below. Dilute the reconstituted 2 ng/ml standard stock according to the chart below. Vortex each tube briefly to ensure complete mixing.

   Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of standard should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Concentration of Standards	Volume of 2 ng/ml Stock to Add	Volume of Assay Buffer to Add
0.01 ng/ml	0.005 ml	0.995 ml
0.04 ng/ml	0.020 ml	0.980 ml
0.10 ng/ml	0.050 ml	0.950 ml
0.25 ng/ml	0.125 ml	0.875 ml
0.50 ng/ml	0.250 ml	0.750 ml
1 ng/ml	0.500 ml	0.500 ml
2 ng/ml	----	----

B. Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Rat/Mouse NPY Quality Control 1 and Quality Control 2 with 0.5 ml distilled or deionized water and gently invert to ensure complete hydration. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ -20°C. Avoid further freeze/thaw cycles.

C. Preparation of Capture and Detection Antibody Mixture

Prior to use, combine the entire contents of Rat/Mouse NPY Capture Antibody (3ml) and Rat/Mouse NPY Detection Antibody (3ml), or at a 1:1 ratio, and invert to mix thoroughly.

VIII. REAGENT PREPARATION

D. DILUTION OF MATRIX SOLUTION (WHEN APPLICABLE)

If samples are diluted with assay buffer for this assay, then dilute provided matrix solution to the same degree with assay buffer. For example, if samples are to be diluted 10-fold with assay buffer, dilute the matrix solution 10-fold with assay buffer also.

IX. RAT/MOUSE NPY ELISA ASSAY PROCEDURE

Pre-warm all reagents to room temperature immediately before setting up the assay. Make necessary dilutions of samples and matrix solution with Assay Buffer, if needed.

1. Dilute the 10X concentrated HRP wash buffer 10 fold by mixing the entire contents of both buffer bottles with 900 ml de-ionized or glass distilled water.

2. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C. Assemble the strips in an empty plate holder and fill each well with 300 µl diluted Wash Buffer. Decant wash buffer and remove the residual amount by inverting the plate and tapping it smartly onto absorbent towels several times. Wash assay plate using this procedure 2 additional times. **Do not let wells dry before proceeding to the next step.** If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

3. Add 20 µl Matrix Solution to Blank, Standards and Quality Control wells (refer to § X. for suggested well orientations). **NOTE: If samples are diluted with assay buffer for measurement, the matrix solution has to be diluted accordingly with assay buffer.**

4. Add 30 µl assay buffer to each of the Blank and sample wells.

5. Add 10 µl assay buffer to each of the Standard and Quality Control wells.

6. Add in duplicate 20 µl NPY Standards in the order of ascending concentrations to the appropriate wells.

7. Add in duplicate 20 µl QC1 and 20 µl QC2 to the appropriate wells.

8. Add sequentially 20 µl of the unknown samples in duplicate to the remaining wells**.**

IX. RAT/MOUSE NPY ELISA ASSAY PROCEDURE (continued)

9. Transfer the Antibody Solution Mixture (1:1 mixture of capture and detection antibody) to a buffer or reagent reservoir and add 50 µl to each well with a multi-channel pipette.

10. Cover the plate with plate sealer and incubate at room temperature for 2 hours on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.

11. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.

12. Wash wells 3 times with diluted Wash Buffer, 300 µl per well per wash. Decant and tap after each wash to remove residual buffer.

13. Add 100 µl Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 min on the micro-titer plate shaker.

14. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid.

15. Wash wells 6 times with diluted Wash Buffer, 300 µl per well per wash. Decant and tap after each wash to remove residual buffer.

16. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for **approximately** 10 to 20 minutes. Blue color should be formed in wells of the NPY standards with intensity proportional to increasing concentrations of NPY.
Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

17. Remove sealer and add 100 µL Stop Solution **[CAUTION: CORROSIVE SOLUTION]** and shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Read absorbance at 450 nm and 590nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units.

Assay Procedure for Rat/Mouse NPY ELISA Kit (Cat. # EZRMNPY-27K)

	Step 1	Step 2	Step 3	Step 4-5	Step 6-8	Step 9	Step 10-12	Step 13	Step 14-15	Step 16		Step 17	
Well #	**Dilute both bottles of 10X HRP Wash Buffer with 900 ml de-ionized water.**	**Wash plate 3X with 300 µl diluted HRP wash buffer. Remove residual buffer by tapping smartly on absorbent towels**	Matrix Solution	Assay Buffer	Standards/QCs/ Samples	Capture/ Detection Ab. Mixture	**Seal, Agitate, Incubate 2 hours at Room Temperature. Wash 3X with 300 µl Wash Buffer.**	Enzyme Solution	**Seal, Agitate, Incubate 30 minutes at Room Temperature . Wash 6X with 300 µl Wash Buffer.**	Substrate	**Seal, Agitate, Incubate 10 - 20 minutes at Room Temperature.**	Stop Solution	**Read Absorbance at 450 nm and 590 nm.**
A1, B1			20 µl	30 µl	--	50 µl		100 µl		100 µl		100 µl	
C1, D1			20 µl	10 µl	20 µl of 0.01 ng/ml Standard								
E1, F1			20 µl	10 µl	20 µl of 0.04 ng/ml Standard								
G1, H1			20 µl	10 µl	20 µl of 0.1 ng/ml Standard								
A2, B2			20 µl	10 µl	20 µl of 0.25 ng/ml Standard								
C2, D2			20 µl	10 µl	20 µl of 0.50 ng/ml Standard								
E2, F2			20 µl	10 µl	20 µl of 1.0 ng/ml Standard								
G2, H2			20 µl	10 µl	20 µl of 2.0 ng/ml Standard								
A3, B3			20 µl	10 µl	20 µl of QC 1								
C3, D3			20 µl	10 µl	20 µl of QC 2								
E3, F3			--	30 µl	20 µl of Sample 1								
G3, H3 Etc.			--	30 µl	20 µl of sample 2								

X. MICROTITER PLATE ARRANGEMENT

Rat/Mouse NPY ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	0.25 ng/ml	QC1	Etc.								
B	Blank	0.25 ng/ml	QC1	Etc.								
C	0.01 ng/ml	0.50 ng/ml	QC2									
D	0.01 ng/ml	0.50 ng/ml	QC2									
E	0.04 ng/ml	1.0 ng/ml	Sample 1									
F	0.04 ng/ml	1.0 ng/ml	Sample 1									
G	0.10 ng/ml	2.0 ng/ml	Sample 2									
H	0.10 ng/ml	2.0 ng/ml	Sample 2									

XI. CALCULATIONS

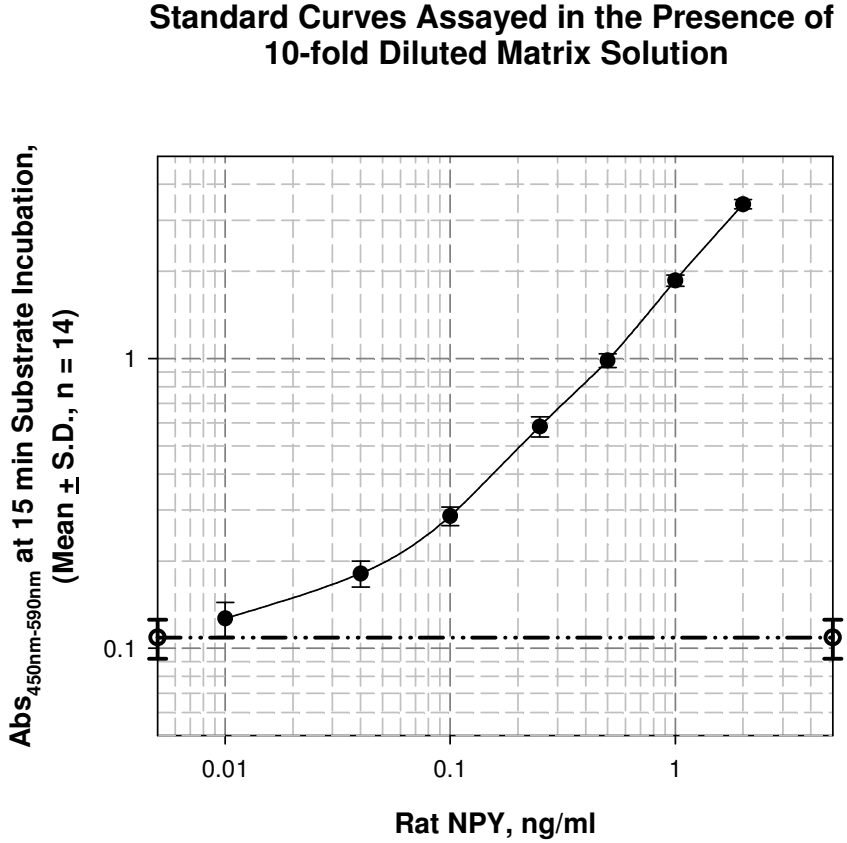
The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function, then multiplied by the sample dilution factor, if any.

Note: When sample volumes assayed differ from 20 µl, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 10 µl of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 20 µl, compensate the volume deficit with matrix solution, with appropriate degree of dilution as all samples used in the assay.

XII. INTERPRETATION

1. The assay will be considered accepted when all Quality Control values fall within the calculated QC range. If any QCs fall outside of the control range, review results with a supervisor.
2. If the difference between duplicate results of a sample is >15% CV, repeat the sample.
3. The theoretical minimal detecting concentration of this assay is 0.002 ng/ml NPY (20 µl sample size, neat or appropriately diluted).
4. The appropriate range of this assay is 0.01 ng/ml to 2 ng/ml NPY (20 µl sample size, neat or appropriately diluted). Any result greater than 2 ng/ml in a 20 µl sample (neat or appropriately diluted) should be diluted using assay buffer and the assay repeated, using matrix solution diluted to the same degree with assay buffer, until the results fall within range.

XIII. GRAPH OF TYPICAL REFERENCE CURVE



Typical Standard Curve, not to be used to calculate data.

XIV. ASSAY CHARACTERISTICS

A. Analytical Sensitivity

The lowest level of Active NPY that can be detected by this assay is 0.002 ng/ml when using a 20 µl sample size, as derived from Statistical Ligand Immunoassay Analysis of multiple assays (n = 14) calculating the mean plus 2 standard deviations of the minimal detectable concentrations.

XIV. ASSAY CHARACTERISTICS (continued)

B. Specificity

Rat, Mouse, Human NPY	100%
Rat, Mouse, Human NPY 2-36	67%
Rat, Mouse, Human NPY 3-36	68%
Rat, Mouse, Human NPY (Free Acid)	6%
Rat, Mouse, Human 1-24	0%
Rat, Mouse, Human 13-36	8%
Rat, Mouse, Human NPY 18-36	0%
NPY 22-36	0%
(Leu^{31}, Pro^{34}) Human, Rat NPY	41%
Porcine NPY	44%
Porcine NPY 3-36	41%
Rat, Mouse, Porcine, Canine, Human PYY	0%
Rat, Mouse, Porcine, Canine, Human PYY 3-36	0%
Rat, Human PP	0%
Rat, Human GIP	0%
Rat, Mouse, Human Ghrelin	0%
Intact Human Proinsulin	0%
Glucagon	0%

C. Precision

Intra- and Inter-Assay Variations (n = 6)

Sample		NPY (ng/ml)	Intra-assay CV (%)	Inter-assay CV (%)
Mouse Plasma	**CD-1**	1.80	1.85	8.58
	Swiss Webster	7.03	1.15	6.16
	C57BL/6	1.00	1.32	5.87
	BALB/C	4.46	1.51	11.6
Rat Plasma	**Sprague Dawley**	4.53	1.57	5.59
	Wistar Hannover	3.55	1.47	7.90
	Fischer 344	9.00	0.85	9.79
	Zucker Lean	2.06	2.68	5.97

Plasma sample of Zucker rat is diluted 40-fold and all others 10-fold in assay buffer for NPY ELISA. The levels of NPY shown are corrected for the dilution factors. Intra-assay variations were calculated from results of 5 duplicate determinations in one assay. Inter-assay variations were calculated from results of 6 separate assays with duplicate samples in each assay.

XIV. ASSAY CHARACTERISTICS (continued)

D. Spike Recovery Rate of NPY in Rat/Mouse Plasma Samples

Plasma Sample	I.D.	Basal NPY	+ NPY (0.1 ng/ml)		+ NPY (0.5 ng/ml)		+ NPY (1.0 ng/ml)	
		ng/ml	ng/ml	Recovery	ng/ml	Recovery	ng/ml	Recovery
Mouse	CD-1	0.130	0.214	84.0 %	0.553	84.6 %	1.028	89.8 %
	Swiss Webster	0.283	0.373	90.0 %	0.704	84.2 %	1.173	89.0 %
	C57BL/6	0.079	0.164	85.0 %	0.494	83.0 %	0.945	86.6 %
	BALB/C	0.482	0.577	95.0 %	0.950	93.6 %	1.433	95.1 %
	OB/OB	0.072	0.163	91.0 %	0.556	96.8 %	0.920	84.8 %
	db/db	0.090	0.191	101.0 %	0.601	102.2 %	0.984	89.4 %
% Recovery Rate (Mean ± S.D., n = 6)		100 %	91.0 % ± 6.36 %		90.7 % ± 7.96 %		89.1 % ± 3.50 %	
Rat	Sprague Dawley	0.217	0.300	83.0 %	0.622	81.0 %	1.089	87.2 %
	Wistar Hannover	0.372	0.454	82.0 %	0.812	88.0 %	1.269	89.7 %
	Fischer 344	0.513	0.611	98.0 %	0.976	92.6 %	1.460	94.7 %
	Zucker Fatty	0.496	0.596	100 %	0.991	99.0 %	1.494	99.8 %
	Zucker Lean	0.077	0.170	93.0 %	0.531	90.8 %	1.008	93.1 %
% Recovery Rate (Mean ± S.D., n = 5)		100 %	91.2 % ± 8.35 %		90.3 % ± 6.58 %		92.9 % ± 4.84 %	

Zucker rat plasma samples are diluted 40-fold and all others 10-fold in assay buffer. NPY at indicated concentrations are then spiked into diluted samples for measurement of recovery rate. NPY levels reported in the Table are not corrected for dilution factors. The recovery rate = [(Observed NPY concentration after spike – Basal NPY level) / spiked NPY concentration] x 100%.

E. Linearity of Sample Dilution

Plasma Sample	I. D.	Sample Volume						
		20 µl	15 µl		10 µl		5 µl	
		ng/ml	ng/ml	Expected	ng/ml	Expected	ng/ml	Expected
Mouse	CD-1	1.99	2.09	105.0 %	2.08	104.5 %	2.28	114.6 %
	Swiss Webster	16.46	16.23	98.6 %	14.94	90.8 %	13.96	84.8 %
	C57BL/6	1.21	1.21	100.3 %	1.26	104.1 %	1.40	115.7 %
	BALB/C	5.54	5.44	98.2 %	5.26	95.0 %	5.24	94.6 %
	OB/OB	1.32	1.40	106.1 %	1.54	116.7 %	1.56	118.2 %
	db/db	2.31	2.44	105.6 %	2.64	114.3 %	2.80	121.2 %
% Expected (Mean ± S.D., n = 6)		100 %	102.3 % ± 3.66 %		104.2 % ± 10.2 %		108.2 % ± 14.8 %	
Rat	Sprague Dawley	3.74	3.77	100.9 %	3.68	98.4 %	4.08	109.1 %
	Wistar Hannover	18.10	18.40	101.7 %	19.68	108.7 %	17.96	99.2 %
	Fischer 344	4.04	4.19	103.6 %	4.08	101.0 %	4.64	114.9 %
	Zucker Fatty	46.24	41.72	90.2 %	41.60	90.0 %	45.60	98.6 %
	Zucker Lean	28.24	27.04	95.8 %	27.92	98.9 %	28.64	101.4 %
% Expected (Mean ± S.D., n = 5)		100 %	98.4 % ± 5.44 %		99.4 % ± 6.68 %		104.6 % ± 7.10 %	

Zucker rat plasma samples are diluted 40-fold and all others 10-fold in assay buffer for NPY ELISA. All diluted samples were assayed at 20, 15, 10 and 5 µl while volumes less than 20 µl are compensated with matrix solution diluted in assay buffer to similar degree as samples being used. Measured NPY levels are corrected for various dilution factors and then divided by levels found at 20 µl sample size to obtain the % of expected values.

XV. QUALITY CONTROLS

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\lincoresearch.

XVI. TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubble formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High absorbance in background or blank wells could be due to 1) cross well contamination by standard solution or sample or 2) inadequate washing of wells with HRP Wash Buffer or 3) overexposure to light after substrate has been added.

XVII. REPLACEMENT REAGENTS

Reagents	**Cat. #**
Microtiter Plates	EPDAR
10X HRP Wash Buffer Concentrate (50 ml)	EWB-HRP
Rat/Mouse NPY Standard	E8027-K
Rat/Mouse NPY Quality Controls 1 and 2	E6027-K
Matrix Solution	EMTX-PS3
Assay Buffer	AB-P
Rat/Mouse NPY Capture Antibody	E1027-C
Rat/Mouse NPY Detection Antibody	E1027-D
Enzyme Solution	EHRP
Substrate	ESS-TMB2
Stop Solution	ET-TMB

XVIII. 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

TELEPHONE ORDERS:

Toll Free US: (800) MILLIPORE

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 human or animals. All products are intended for *in vitro* use only.

C. Material Safety Data Sheets (MSDS)

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