



Human NPY
96-Well Plate Assay
Cat. # EZHNPY-25K

HUMAN NPY ELISA KIT
96-Well Plate (Cat. # EZHNPY-25K)

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HUMAN NPY ELISA Kit
96-Well Plate (Cat.# EZHNPY-25K)

I. INTENDED USE

This kit is used for the non-radioactive quantification of Neuropeptide Y (NPY) in human serum and plasma. One kit is sufficient to measure 38 unknown samples in duplicate. ***This kit is for Research Use Only. Not for Use in Diagnostic Procedures.***

II. PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based on: 1) capture of NPY in the sample by anti-human 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) binding of a second biotinylated antibody to NPY after brief washings, 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 450 nm, 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 Human 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 50 mL each

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

4. Human NPY Standard

Human NPY reference standard, lyophilized

Quantity: 1 bottle, 2 mL/bottle upon hydration

Preparation: Hydrate thoroughly in distilled or de-ionized water immediately before use. Please refer to the analysis sheet for exact concentration.

5. Human NPY Quality Controls 1 and 2

One vial each, lyophilized, containing human 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: 1.5 mL/vial

Preparation: Ready to use.

7. Assay Buffer

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

Quantity: 20 mL/vial

Preparation: Ready to use.

III. REAGENTS SUPPLIED (CONTINUED)

8. Human NPY Capture Antibody

Pre-titered capture antibody solution in buffer

Quantity: 3 mL/vial

Preparation: Ready to use.

9. Human NPY Detection Antibody

Pre-titered detection antibody solution in buffer

Quantity: 12 mL/vial

Preparation: Ready to use.

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

Recommended storage for kit components is 2-8°C.

All components are shipped and stored at 2-8°C. Reconstituted standards and quality controls can be frozen for future use, but repeated freeze thaws should be avoided. 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 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

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.

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

Full labels of hazardous components in this kit:

Ingredient, Cat #		Full Label	
Human Neuropeptide Y Capture Antibody	E1025-C		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 Neuropeptide Y Detection Antibody	E1025-D		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 Neuropeptide Y Quality Controls 1 & 2	E6025-K	 	Danger. Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention.
Human Neuropeptide Y Standard	E8025-K	 	Danger. Harmful if swallowed. Causes serious eye damage. Harmful to aquatic life with long lasting effects. Avoid release to the environment. Wear eye protection. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/attention.
Stop Solution	ET-TMB		Warning. May be corrosive to metals.
10X HRP Wash Buffer Concentrate	EWB-HRP		Warning. May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipettes and pipette tips: 10 μ L ~ 20 μ L or 20 μ L ~ 100 μ L
2. Multi-channel Pipettes and pipette tips: 5 ~ 50 μ L and 50 ~ 300 μ L
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. De-ionized Water
6. Microtiter Plate Reader capable of reading absorbency at 450 nm and 590 nm
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 EMD Millipore Cat# DPP4-010) is required for Sample Collection and Storage.

VII. SAMPLE COLLECTION AND STORAGE

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

1. To prepare serum, whole blood is directly drawn into a centrifuge tube that contains no anti-coagulant. Immediately add enough DPP-IV Inhibitor and Aprotinin to a final concentration of 50 μ M and 500 KIU/mL, respectively. Let blood clot at room temperature for 30 min.
2. Promptly centrifuge the clotted blood at 2,000 to 3,000 x g for 15 minutes at 4 \pm 2 $^{\circ}$ C.
3. Transfer and aliquot serum samples in separate tubes of small quantity. Date and identify each sample.
4. Use freshly prepared serum or store samples at -20 \pm 5 $^{\circ}$ C for later use. Avoid multiple (> 5) freeze/thaw cycles.
5. To prepare plasma sample, whole blood should be collected into a centrifuge tube containing enough K₃ EDTA to achieve a final concentration of 1.735 mg/mL and treated with DPP-IV Inhibitor and Aprotinin as described for serum, followed by immediate centrifugation. Observe same precautions in the preparation of serum samples.
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.

VIII. REAGENT PREPARATION

A. Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the Human NPY Standard with 2 mL of deionized water. Please refer to the analysis sheet for exact concentration. Invert and mix gently until completely in solution.
2. Label six tubes 1, 2, 3, 4, 5, and 6. Add Assay Buffer to each of the six tubes according to the volumes outlined in the chart below. Dilute the reconstituted 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 $\leq -20^{\circ}\text{C}$. Avoid multiple freeze/thaw cycles.

Volume of Deionized Water to Add	Volume of Standard to Add	Standard Concentration pg/mL
2 mL	0	X (refer to analysis sheet For exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (pg/mL)
1	500 μL	500 μL of reconstituted Standard	X/2
2	500 μL	500 μL of Tube 1	X/4
3	500 μL	500 μL of Tube 2	X/8
4	500 μL	500 μL of Tube 3	X/16
5	500 μL	500 μL of Tube 4	X/32
6	500 μL	500 μL of Tube 5	X/64

B. Quality Control 1 and 2 Preparation

Use care in opening the lyophilized Quality Control vials. Reconstitute each Human 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 $\leq -20^{\circ}\text{C}$. Avoid further freeze/thaw cycles.

IX. HUMAN NPY ELISA ASSAY PROCEDURE

Pre-warm all reagents to room temperature immediately before setting up the assay.

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 μ L 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 50 μ L Matrix Solution to Blank, Standards and Quality Control wells (refer to § X. for suggested well orientations).
4. Add 50 μ L assay buffer to each of the Blank and sample wells.
5. Add in duplicate 50 μ L NPY Standards in the order of ascending concentrations to the appropriate wells.
6. Add in duplicate 50 μ L QC1 and 50 μ L QC2 to the appropriate wells.
7. Add sequentially 50 μ L of the unknown samples in duplicate to the remaining wells.
8. Add 20 μ L Capture Antibody Solution to each well with a multi-channel pipette.
9. 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.
10. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well.
11. Wash wells 3 times with diluted Wash Buffer, 300 μ L per well per wash. Decant and tap after each wash to remove residual buffer.
12. Add 100 μ L Detection Antibody Solution to each well. Re-cover plate with sealer and incubate with moderate shaking at room temperature for 1 hour on the microtiter plate shaker.

IX. HUMAN NPY ELISA ASSAY PROCEDURE (continued)

13. Remove plate sealer and decant solutions from the plate. Tap as before to remove residual solutions in well. Wash wells 3 times with diluted Wash Buffer, 300 μ L per well per wash. Decant and tap after each wash to remove residual buffer.
14. Add 100 μ L 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.
15. Remove sealer, decant solutions from the plate and tap plate to remove the residual fluid.
16. Wash wells 6 times with diluted Wash Buffer, 300 μ L per well per wash. Decant and tap after each wash to remove residual buffer.
17. Add 100 μ L 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.

18. Remove sealer and add 100 μ L 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. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. 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 Human NPY ELISA Kit (Cat. # EZHNPY-25K)

	Step 1	Step 2	Step 3	Step 4	Step 5-7	Step 8	Step 9-11	Step 12	Step 13	Step 14	Step 15-16	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 Antibody Solution	Seal, Agitate, Incubate 2 hours at Room Temperature. Wash 3X with 300 µL Wash Buffer.	Detection Antibody Solution	Seal, Agitate, Incubate 1 hour 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			50 µL	50 µL	--	20 µL		100 µL		100 µL		100 µL			
C1, D1			50 µL	--	50 µL of Tube 6 Std	↓		↓		↓		↓			
E1, F1			50 µL	--	50 µL of Tube 5 Std										
G1, H1			50 µL	--	50 µL of Tube 4 Std										
A2, B2			50 µL	--	50 µL of Tube 3 Std										
C2, D2			50 µL	--	50 µL of Tube 2 Std										
E2, F2			50 µL	--	50 µL of Tube 1 Std										
G2, H2			50 µL	--	50 µL of Reconstituted Std										
A3, B3			50 µL	--	50 µL of QC 1										
C3, D3			50 µL	--	50 µL of QC 2										
E3, F3			--	50 µL	50 µL of Sample 1										
G3, H3 Etc.			--	50 µL	50 µL of sample 2										

X. MICROTITER PLATE ARRANGEMENT

Human NPY ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 3 Std	QC1	Etc.								
B	Blank	Tube 3 Std	QC1	Etc.								
C	Tube 6 Std	Tube 2 Std	QC2									
D	Tube 6 Std	Tube 2 Std	QC2									
E	Tube 5 Std	Tube 1 Std	Sample 1									
F	Tube 5 Std	Tube 1 Std	Sample 1									
G	Tube 4 Std	Reconstituted Std	Sample 2									
H	Tube 4 Std	Reconstituted Std	Sample 2									

XI. CALCULATIONS

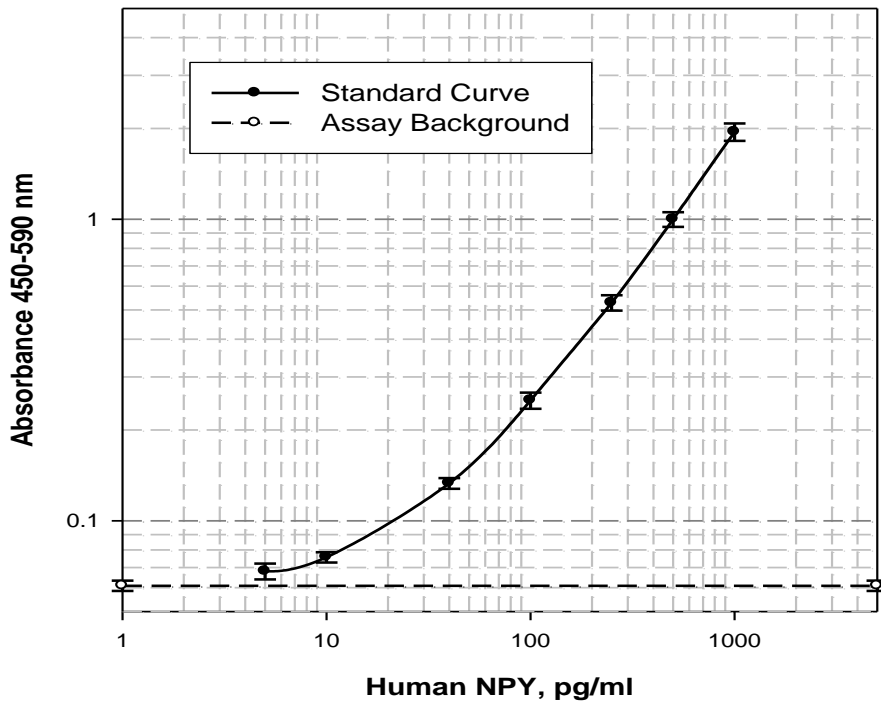
The dose-response curve of this assay fits best to a sigmoidal 4- or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4- or 5-parameter logistic function

Note: When sample volumes assayed differ from 50 μ l, an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 25 μ L of sample is used, then calculated data must be multiplied by 2). When sample volume assayed is less than 50 μ l, compensate the volume deficit with matrix solution.

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 limit of sensitivity of this assay is 2 pg/mL NPY (50 μ L sample size).
4. The appropriate range of this assay is 5 pg/mL to 1,000 pg/ml NPY (50 μ L sample size). Any result greater than 1,000 pg/mL in a 50 μ L sample should be diluted using matrix solution and the assay repeated 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. Sensitivity

The lowest level of NPY that can be detected by this assay is 2 pg/mL using a 50 μ L sample size, as derived from Statistical Ligand Immunoassay Analysis of multiple assays (n = 6) calculating the mean plus 2 standard deviations of the minimal detectable concentrations.

XIV. ASSAY CHARACTERISTICS (continued)

B. Specificity

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

C. Precision

Intra and Inter-Assay Variations

Sample ID	NPY (pg/ml) Mean, n = 5	Intra-assay CV (%)	Inter-assay CV (%)
Serum #18	19.6	3.89	8.12
Serum # 3	25.4	2.06	6.58
Serum #13	41.2	2.60	2.92
Serum # 2	47.1	4.27	4.46
Plasma # 6	16.8	3.84	9.40
Plasma # 12	19.9	3.28	8.82
Plasma # 15	26.7	3.66	13.5
Plasma # 14	35.8	3.13	8.62

Human serum and plasma samples in small aliquots frozen at -70°C are assayed for NPY. Intra-assay variations were calculated from results of five duplicate determinations in one assay. Inter-assay variations were calculated from results of five separate assays with duplicate samples in each assay.

XIV. ASSAY CHARACTERISTICS (continued)

D. Spike Recovery Rate of Human NPY in Assay Samples

Sample	I.D.	Basal NPY	Basal + 40 pg/mL Spiked NPY		Basal + 500 pg/mL Spiked NPY	
		pg/mL	pg/mL	Recovery	pg/mL	Recovery
Serum	# 19	12.8	51.4	96.5 %	501.4	97.6 %
	# 20	16.4	51.4	87.5 %	417.0	80.1 %
	# 25	27.7	73.3	114.0%	556.5	105.8 %
	# 26	141.6	180.6	97.5 %	636.6	99.0 %
% Recovery Rate Mean ± S.D.	----	100 %	98.9 % ± 11.1 %		95.6 % ± 11.0 %	
Plasma	# 19	5.9	45.1	98.0 %	503.4	99.5 %
	# 2	26.4	65.1	96.8 %	553.2	105.4 %
	# 15	10.1	45.5	88.5 %	479.7	93.9 %
	# 23	26.8	63.8	92.5 %	519.9	98.6 %
% Recovery Rate Mean ± S.D.	----	100 %	94.0 % ± 4.3 %		95.6 % ± 11.0 %	

Varying amounts of human NPY were added to individual human serum and plasma samples and the resulting NPY content of each sample was assayed by Human NPY ELISA. The recovery rate = [(Observed NPY concentration after spike – Basal NPY level) / spiked NPY concentration] x 100%.

XIV. ASSAY CHARACTERISTICS (continued)

E. Linearity of Sample Dilution

Sample	I. D.	Sample Volume						
		50 μ L	30 μ L		20 μ L		10 μ L	
		pg/mL	pg/mL	Expected	pg/mL	Expected	pg/mL	Expected
Serum	# 19	651	389	100%	264	102%	132	101%
	# 20	292	179	103%	120	103%	64.4	110%
	# 25	249	146	98%	98.8	99 %	48.8	98%
	# 26	153	91.2	99%	60.1	98 %	24.8	81%
% Expected Mean \pm S.D.	----	100 %	99.8 % \pm 2.0 %		100.4 % \pm 2.3 %		97.7 % \pm 12.3 %	
Plasma	# 19	565	350	103%	229	101%	116	102%
	# 2	352	204	96 %	131	93%	64.4	92%
	# 15	198	120	101%	80.8	102%	40.8	103%
	# 23	131	69.3	88%	44.8	85 %	22.1	84%
% Expected Mean \pm S.D.	----	100 %	97.1 % \pm 6.7 %		95.3 % \pm 7.9 %		95.3 % \pm 9.8 %	

Samples are spiked with human NPY to various levels and assayed by ELISA at the indicated volumes supplemented with Matrix Solution to a total of 50 μ l per well. Measured NPY levels are corrected for various dilution factors and then divided by levels found at 50 μ l sample size to obtain the % of expected values.

XV. NORMAL RANGE OF NPY LEVELS IN HUMAN BLOOD

The normal range of serum/plasma NPY in healthy human subjects has been reported to be within the range 5 ~ 90 pg/mL in the literatures

XVI. QUALITY CONTROLS

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.

XVII. 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.

XVIII. REPLACEMENT REAGENTS

Reagents

	Cat. #
Microtiter Plates	EPDAR
10X HRP Wash Buffer Concentrate (50 ml)	EWB-HRP
Human NPY Standard	E8025-K
Human NPY Quality Controls 1 and 2	E6025-K
Matrix Solution	EMTXD4
Assay Buffer	AB-PHK
Human NPY Capture Antibody	E1025-C
Human NPY Detection Antibody	E1025-D
Enzyme Solution	EHRP
Substrate	ESS-TMB2
Stop Solution	ET-TMB

XIX. 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.