Human Leptin

96-Well Plate Assay

Cat. # EZHL-80SK

HUMAN LEPTIN ELISA KIT
96-Well Plate (Cat. # EZHL-80SK)

Millipore • 6 Research Park Drive • St. Charles, Missouri 63304 USA
Phone: Toll Free U.S.: 800-MILLIPORE • Fax: 636-441-8050
techserve_dd@millipore.com • www.millipore.com/bmia

HUMAN LEPTIN ELISA KIT
96-Well Plate (Cat. # EZHL-80SK)

I. INTENDED USE

This kit is used for the non-radioactive quantification of human leptin in serum, plasma and other biological media. One kit is sufficient to measure 37 unknown samples in duplicate. ***This kit is for research purposes only.***

II. PRINCIPLES OF PROCEDURE[1]

This assay is a direct Sandwich ELISA based, sequentially, on: 1) capture of human leptin by a polyclonal rabbit anti-human leptin antibody immobilized on a 96-well microtiter plate, 2) wash away unbound materials, 3) binding of a biotinylated monoclonal antibody to the captured human leptin, 4) wash away unbound materials, 5) binding of streptavidin-horseradish peroxidase to the immobilized biotinylated antibodies, 6) wash away free enzyme conjugates, and 7) quantification of bound streptavidin-horseradish peroxidase with the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbency at 450 nm - 590nm after acidification of formed products. Since the increase in absorbency is directly proportional to the amount of captured human leptin in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of human leptin.

III. REAGENTS SUPPLIED

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

A. Human Leptin ELISA Plate
Coated with Rabbit anti-Human Leptin Antibody
Quantity: 1 plate
Preparation: Ready to use
Note: Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C.

B. Adhesive Plate Sealer
Quantity: 2 Sheets
Preparation: Ready to use

C. 10X HRP Wash Buffer Concentrate
10X concentrate of 50 mM Tris Buffered Saline containing Tween 20
Quantity: Two bottles containing 50 mL each
Preparation: Dilute 1:10 with deionized water

III. REAGENTS SUPPLIED (continued)

D. Human Leptin ELISA Standards

Human Leptin in Assay Buffer: 0.5, 1, 2, 5, 10, 20, 50 and 100 ng/mL
Quantity: 1 mL/vial
Preparation: Ready to use

E. Quality Controls 1 and 2

Purified Recombinant Human Leptin in QC Buffer
Quantity: 0.5 mL/vial
Preparation: Ready to use

F. Assay Buffer

0.05M PBS, pH 7.4, containing 0.025M EDTA, 0.08% Sodium Azide, 1% BSA and 0.05% Triton X-100
Quantity: 10 mL/vial
Preparation: Ready to use

G. Human Leptin Detection Antibody

Biotinylated Mouse anti-Human Leptin Antibody
Quantity: 11 mL/vial
Preparation: Ready to use

H. Enzyme Solution

Streptavidin-Horseradish Peroxidase Conjugate in Buffer
Quantity: 12 mLl/vial
Preparation**:** Ready to use

I. Substrate

3,3'5,5'-tetramethylbenzidine
Quantity: 12 mL
Preparation: Ready to use

J. Stop Solution

0.3M HCL
Quantity: 12 mL/vial
Preparation: Ready to use **(Caution: Corrosive Material)**

IV. STORAGE AND STABILITY

Upon receipt, all components of the kit should be stored at 2-8°C. For prolonged storage (>2 weeks), store the Wash Buffer, Assay Buffer, Standards, and Controls at ≤ -20°C and store the Detection Antibody, Enzyme Solution, Substrate, and Plate at 2-8°C. Unused 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

A. Hydrochloric Acid

Hydrochloric Acid is corrosive and can cause eye and skin burns. It is harmful if swallowed and can cause respiratory and digestive tract burns. Avoid contact with skin and eyes. Do not swallow or ingest.

B. Sodium Azide

Sodium Azide has been added to some 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 large volume of water to prevent azide build up.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Pipette with Tips, 10µL-200µL
2. Multi-channel Pipette, 50µL-300µL
3. Buffer and Reagent Reservoirs
4. Vortex Mixer
5. Absorbent Paper or Cloth
6. Deionized Water
7. Microtiter Plate Reader capable of reading absorbency at 450 nm
8. Orbital Microtiter Plate Shaker

VII. SAMPLE COLLECTION AND STORAGE

1. To prepare serum, whole blood is directly drawn into a Vacutainer® serum tube that contains no anti-coagulant. Let blood clot at room temperature for 30 minutes.

2. Promptly centrifuge the clotted blood at 2000 to 3000 x.g. for 15 minutes at 4 ± 2ºC.

3. Transfer and store serum samples in separate tubes. Date and identify each sample.

4. Use freshly prepared serum or store samples at ≤ -20°C for later use. Avoid multiple (>5) freeze/thaw cycles.

5. To prepare plasma samples, whole blood should be collected into Vacutainer® EDTA-plasma tubes and centrifuged immediately after collection. Observe same precautions in the preparation of serum samples.

6. If heparin is to be used as an 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. ASSAY PROCEDURE I
(Sensitivity: 0.5 ng/mL – 100 ng/mL)

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

1. Dilute the concentrated Wash Buffer 10 fold by adding the entire contents of both bottles of buffer to 900 mL de-ionized or glass distilled water.

2. Remove required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C. Assemble strips in an empty plate holder and add 300 µL of diluted Wash Buffer to each well. Incubate at room temperature for 5 minutes. Decant wash buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several 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 75 µL Assay Buffer into all wells.

4. Add in duplicate 25 µL Assay Buffer to blank wells. (Refer to Section IX for suggested well Orientations.)

5. Add in duplicate 25 µL Human Leptin Standards in order of ascending concentration to the appropriate wells. Add in duplicate 25 µL QC1 and 25 µL QC2 to the appropriate wells. Add sequentially 25 µL of samples in duplicate to the remaining wells**. For best results all additions should be completed within one hour**.

6. 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.

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

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

9. Add 100 µL Detection Antibody to each well. Cover the plate with sealer and incubate at room temperature for 30 minutes on the microtiter plate shaker.

10. Remove sealer and decant solution from the plate. Tap as before to remove residual solutions in the wells.

11. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

12. Remove sealer, decant solution from the plate, and tap plate to remove the residual fluid.

VIII. ASSAY PROCEDURE I (continued)

13. Wash wells 5 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

14. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for 5-20 minutes. Blue color should be formed in wells of Leptin standards with intensity proportional to increasing concentrations of Leptin.

    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. One can monitor color development using 370 nm filter, if available on the spectrophotometer. When the absorbance is between 1.2 and 1.8 at 370 nm, the stop solution can be added to terminate the color development.

15. Remove sealer and add 100 µL of 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 450nm and 590nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference in absorbance units.

Assay Procedure I for Human Leptin ELISA kit (Cat. # EZHL-80SK)

	Step 1	Step 2	Step3-4	Step 5	Step 6-8	Step 9	Step 9-10	Step 11	Step 11-13	Step 14	Step 14	Step 15	Step 15
Well #	Dilute 2 bottles of 10X Wash Buffer with 900mL Deionized Water.	Wash plate with 300 µlL Wash Buffer and incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels	Assay Buffer	Standards/ Controls/ Samples	Seal, Agitate, Incubate 2 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer	Detection Ab	Seal, Agitate, Incubate 30 minutes at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels	Enzyme Solution	Seal, Agitate, Incubate 30 minutes at Room Temperature . Wash 5X with 300 µl Wash Buffer	Substrate	Seal, Agitate, Incubate 5-20 minutes at Room Temperature.	Stop Solution	Read Absorbance at 450 nm and 590 nm.
A1, B1			100 µL	---		100 µL		100 µL		100 µL		100 µl	
C1, D1			75 µL	25 µL of 0.5 ng/mL Standard									
E1, F1				25 µL of 1.0 ng/mL Standard									
G1, H1				25 µL of 2.0 ng/mL Standard									
A2, B2				25 µL of 5.0 ng/mL Standard									
C2, D2				25 µL of 10 ng/mL Standard									
E2, F2				25 µL of 20 ng/mL Standard									
G2, H2				25 µL of 50 ng/mL Standard									
A3, B3				25 µL of 100 ng/mL Standard									
C3, D3				25 µL of QC I									
E3, F3				25 µL of QC II									
G3, H3				25 µL of Sample									
A4, B4 ↓				25 µL of Sample									

IX. MICROTITER PLATE ARRANGEMENT – ASSAY PROCEDURE I

Human Leptin ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	5.0 ng/mL	100 ng/mL	Sample 2								
B	Blank	5.0 ng/mL	100 ng/mL	Sample 2								
C	0.5 ng/mL	10.0 ng/mL	QC 1	Etc.								
D	0.5 ng/mL	10.0 ng/mL	QC 1									
E	1.0 ng/mL	20.0 ng/mL	QC 2									
F	1.0 ng/mL	20.0 ng/mL	QC 2									
G	2.0 ng/mL	50 ng/mL	Sample 1									
H	2.0 ng/mL	50 ng/mL	Sample 1									

X. ASSAY PROCEDURE II – Human Leptin (Sensitive) Assay (Sensitivity: 0.125 ng/mL – 20 ng/mL)

A. Human Leptin (Sensitive) Standard Preparation

Note: The standard curve range recommended for samples with low Leptin levels is 0.125ng/mL to 20ng/mL. By performing 1:1 serial dilutions of the 0.5ng/mL kit standard with assay buffer, the 0.25ng/mL and 0.125ng/mL standard points can be created for use in the Leptin Sensitive Assay.

1. Label two tubes 0.25 and 0.125 ng/mL. Add 0.2mL Assay Buffer to each of the two tubes. Prepare serial dilutions by adding 0.2 mL of the 0.5 ng/mL standard to the 0.25 ng/mL tube, mix well and transfer 0.2 mL of the 0.25 ng/mL standard to the 0.125 ng/mL tube 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°C. Avoid multiple freeze/thaw cycles.

Standard Concentration ng/mL	Volume of Assay Buffer to Add	Volume of Standard to Add
0.25	0.2 mL	0.2 mL of 0.5 ng/mL
0.125	0.2 mL	0.2 mL of 0.25 ng/mL

B. Human Leptin (Sensitive) Quality Control 1 and 2 Preparation

1. Prepare 1:3 dilutions of Quality Control 1 and Quality Control 2 for use in the Human Leptin (Sensitive) assay. Label two tubes QC1 and QC2. Add 0.4mL Assay Buffer to each of the tubes. Add 0.2mL Quality Control 1 to the QC1 tube and mix well. Add 0.2mL Quality Control 2 to the QC2 tube and mix well.

Note: Do not use a Repeater pipette. Change tip for every dilution. Wet tip with Quality Control before dispensing. Unused portions of Quality Control should be stored at ≤ -20°C. Avoid multiple freeze/thaw cycles.

	Volume of Assay Buffer to Add	Volume of Quality Control to Add
QC1	0.4 mL	0.2 mL of Quality Control 1
QC2	0.4 mL	0.2 mL of Quality Control 2

C. ASSAY PROCEDURE II – Human Leptin (Sensitive) Assay (Sensitivity: 0.125 ng/mL – 20 ng/mL)

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

1. Dilute the concentrated Wash Buffer 10 fold by adding the entire contents of both bottles of buffer to 900 mL de-ionized or glass distilled water.

2. Remove required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch with the desiccant provided and stored at 2-8°C. Assemble strips in an empty plate holder and add 300 µL of diluted Wash Buffer to each well. Incubate at room temperature for 5 minutes. Decant wash buffer and remove the residual amount from all wells by inverting the plate and tapping it smartly onto absorbent towels several 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 µL Assay Buffer into all wells.

4. Add in duplicate 50 µL Assay Buffer to blank wells. (Refer to Section XI for suggested well orientations.)

5. Add in duplicate 50 µL Human Leptin Standards in order of ascending concentration to the appropriate wells. Add in duplicate 50 µL QC1 and 50 µL QC2 to the appropriate wells. Add sequentially 50 µL of samples in duplicate to the remaining wells**. For best results all additions should be completed within one hour**.

6. 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.

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

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

9. Add 100 µL Detection Antibody to each well. Cover the plate with sealer and incubate at room temperature for 30 minutes on the microtiter plate shaker.

10. Remove sealer and decant solution from the plate. Tap as before to remove residual solutions in the wells.

11. Add 100 µL Enzyme Solution to each well. Cover plate with sealer and incubate with moderate shaking at room temperature for 30 minutes on the microtiter plate shaker.

C. Assay Procedure II – Human Leptin (Sensitive) Assay (continued)

12. Remove sealer, decant solution from the plate, and tap plate to remove the residual fluid.

13. Wash wells 5 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap firmly after each wash to remove residual buffer.

14. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 5-20 minutes. Blue color should be formed in wells of Leptin standards with intensity proportional to increasing concentrations of Leptin.

    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. One can monitor color development using 370 nm filter, if available on the spectrophotometer. When the absorbance is between 1.2 and 1.8 at 370 nm, the stop solution can be added to terminate the color development.

15. Remove sealer and add 100 µL of 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 450nm and 590nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference in absorbance units.

Assay Procedure II for Human Leptin (Sensitive) ELISA kit (Cat. # EZHL-80SK)

	Step 1	Step 2	Step 3-4	Step 5	Step 6-8	Step 9	Step 9-10	Step 11	Step 11-13	Step 14	Step 14	Step 15	Step 15
Well #	**Dilute 2 bottles of 10X Wash Buffer with 900mL Deionized Water.**	**Wash plate with 300 µL Wash Buffer and incubate at room temperature for 5 minutes. Remove residual buffer by tapping smartly on absorbent towels**	Assay Buffer	Standards/ Controls/ Samples	**Seal, Agitate, Incubate 2 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer**	Detection Ab	**Seal, Agitate, Incubate 30 minutes at Room Temperature. Remove residual buffer by tapping smartly on absorbent towels**	Enzyme Solution	**Seal, Agitate, Incubate 30 minutes at Room Temperature . Wash 5X with 300 µL Wash Buffer**	Substrate	**Seal, Agitate, Incubate 5-20 minutes at Room Temperature.**	Stop Solution	**Read Absorbance at 450 nm and 590 nm.**
A1, B1			100 µL	---		100 µL		100 µL		100 µL		100 µL	
C1, D1			50 µL	50 µL of 0.125 ng/mL Standard									
E1, F1				50 µL of 0.25 ng/mL Standard									
G1, H1				50 µL of 0.5 ng/mL Standard									
A2, B2				50 µL of 1.0 ng/mL Standard									
C2, D2				50 µL of 2.0 ng/mL Standard									
E2, F2				50 µL of 5.0 ng/mL Standard									
G2, H2				50 µL of 10 ng/mL Standard									
A3, B3				50 µl of 20 ng/ml Standard									
C3, D3				50 µL of diluted QC I									
E3, F3				50 µL of diluted QC II									
G3, H3				50 µL of Sample									
A4, B4 ↓				50 µL of Sample									

XI. MICROTITER PLATE ARRANGEMENT II

Human Leptin (Sensitive) ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	1.0 ng/mL	20.0 ng/mL	Sample 2								
B	Blank	1.0 ng/mL	20.0 ng/mL	Sample 2								
C	0.125 ng/mL	2.0 ng/mL	QC 1	Etc.								
D	0.125 ng/mL	2.0 ng/mL	QC 1									
E	0.25 ng/mL	5.0 ng/mL	QC 2									
F	0.25 ng/mL	5.0 ng/mL	QC 2									
G	0.5 ng/mL	10.0 ng/mL	Sample 1									
H	0.5 ng/mL	10.0 ng/mL	Sample 1									

XII. CALCULATIONS

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

Note: When sample volumes assayed differ from 25 µL (In normal assay), an appropriate mathematical adjustment must be made to accommodate for the dilution factor (e.g., if 12.5 µL of sample is used, then calculated data must be multiplied by 2).

XIII. INTERPRETATION

1. The assay should be rejected if one of the two QCs falls outside of 2 standard deviations of the applicable mean. See the supervisor.

2. The limit of sensitivity of this assay is 0.5 ng/mL human leptin (25 µL sample size).

3. The appropriate range of this assay is 0.5 to 100 ng/mL human leptin (25 µL sample size). Any result greater than 100 ng/mL in a 25 µL sample assayed should be diluted and repeated using assay buffer as diluent until it falls within range.

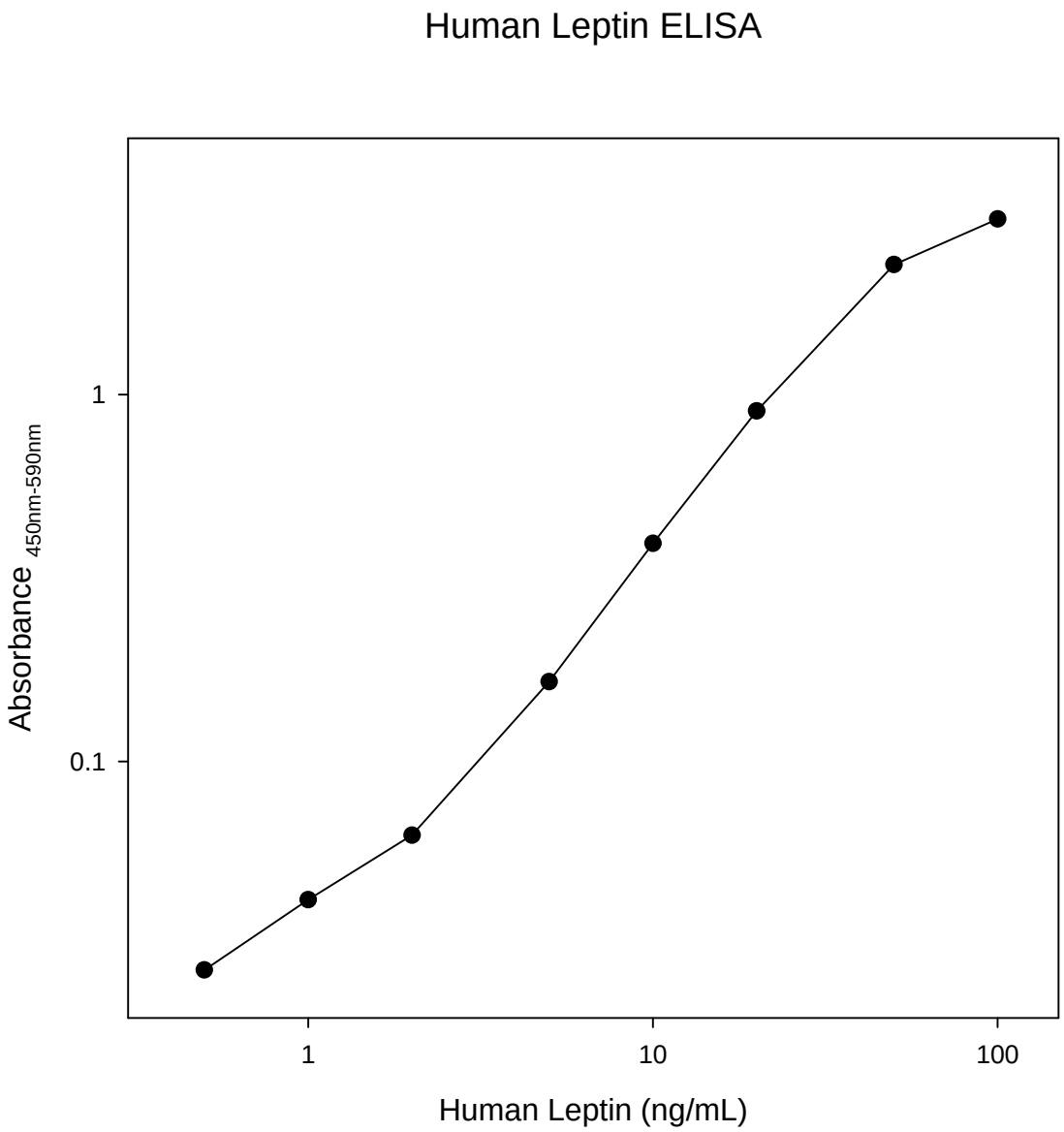
XIV. NORMAL RANGE

Normal range: Leptin levels are directly correlated with degree of adiposity.

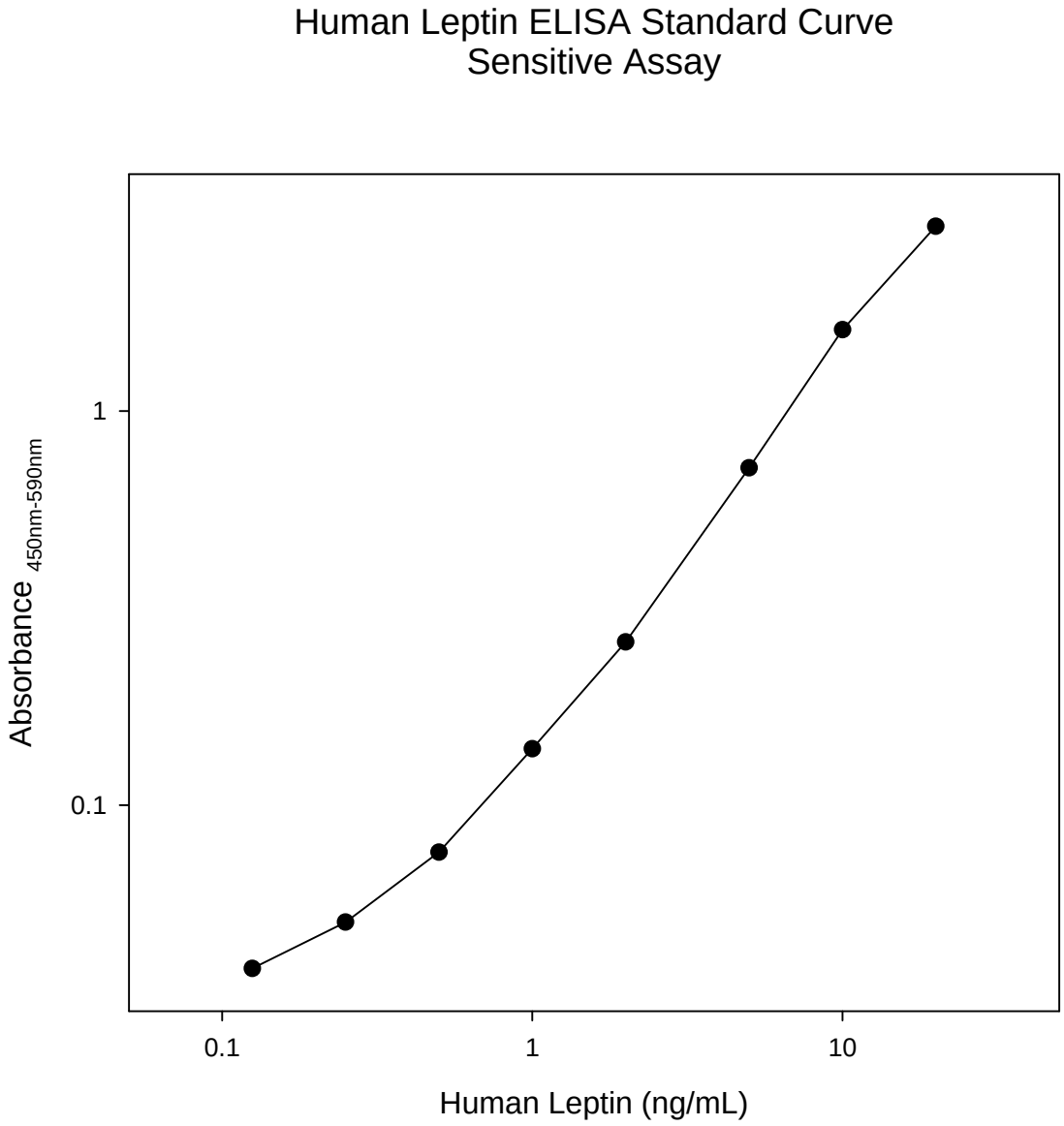
Mean Serum Leptin levels from normal lean individuals (BMI ranges 18-25):
Lean Men 3.8 ± 1.8 ng/mL
Lean Women 7.4 ± 3.7 ng/mL

Serum levels are approximately 2.5 times higher in women per unit BMI as compared to men.

XV. STANDARD CURVE



Typical Standard Curve – Not to be used to calculate data



Typical Standard Curve – Not to be used to calculate data

XVI. ASSAY CHARACTERISTICS

A. Sensitivity

The lowest level of human leptin that can be detected by this assay is 0.5 ng/mL (25 μL sample size). For the sensitive assay, the lowest level of human leptin that can be detected is 0.125 ng/mL (50 μL sample size).

B. Specificity

Human Leptin	100%
Chicken Leptin	n.d.*
Mouse Leptin	n.d.*
Ovine Leptin	n.d.*
Porcine Leptin	n.d.*
Rat Leptin	n.d.*
Bovine Proinsulin	n.d.*
Human Proinsulin	n.d.**
Porcine Proinsulin	n.d.*
Human Insulin	n.d.*
Human IGF-I	n.d.***
Human IGF-II	n.d.***
Glucagon	n.d.*

n.d.: Not detectable at concentrations up to * 625 nM; ** 400 nM and *** 200 nM.

C. Precision

Within and Between Assay Variation

Sample No.	Mean Leptin Levels ng/mL	Within % CV	Between % CV
1	2.34	4.6	6.2
2	9.53	3.4	4.2
3	22.08	4.1	3.0
4	28.9	2.6	2.6

Sensitive Assay

Sample No.	Mean Leptin Levels ng/mL	Within % CV	Between % CV
1	0.86	4.9	8.6
2	4.59	2.2	3.4
3	11.26	1.4	1.7
4	14.08	1.9	1.3

The assay variations of Millipore Human Leptin ELISA kits were studied on four human serum samples with varying concentrations of endogenous leptin. The mean within variations of four duplicate determinations in each assay of the indicated samples were calculated from results of three separate assays. The mean between variation of each sample was calculated from results of three separate assays with four duplicate samples in each assay.

XVI. ASSAY CHARACTERISTICS (continued)

D. Recovery

Spike & Recovery of Human Leptin in Serum

Varying amounts of human leptin were added to three human serum samples and the leptin content was determined in three separate assays.
The % of recovery = observed leptin concentrations/expected leptin concentrations x 100%.

Sample No.	Leptin Added ng/mL	Expected ng/mL	Observed ng/mL	% of Recovery
1	0	4.4	4.4	100
	5	9.4	9.84	104.7
	20	24.4	27.2	111.5
	50	54.4	51.89	95.4
2	0	12.02	12.02	100
	5	17.02	18.91	111.1
	20	32.02	36.54	114.1
	50	62.02	61.61	99.3
3	0	32.79	32.79	100
	5	37.79	39.95	105.7
	20	52.79	52.79	100
	50	82.79	94.33	113.9

Sensitive Assay				
Sample No.	Leptin Added ng/mL	Expected ng/mL	Observed ng/mL	% of Recovery
1	0	0.87	0.87	100.0
	1	1.87	1.8	96.3
	5	5.87	5.7	97.1
	10	10.87	10.4	95.7
2	0	3.59	3.59	100.0
	1	4.59	4.58	99.8
	5	8.59	8.78	102.2
	10	13.59	12.98	95.5
3	0	7.21	7.21	100.0
	1	8.21	8.15	99.3
	5	12.21	11.53	94.4
	10	17.21	14.83	86.2

XVI. ASSAY CHARACTERISTICS (continued)

E. Linearity

Effect of Serum Dilution

Three human serum samples with the indicated sample volumes were assayed in three separate experiments. Required amounts of assay buffer were added to compensate for lost volumes below 25 µL.
% expected = observed/expected x 100%.

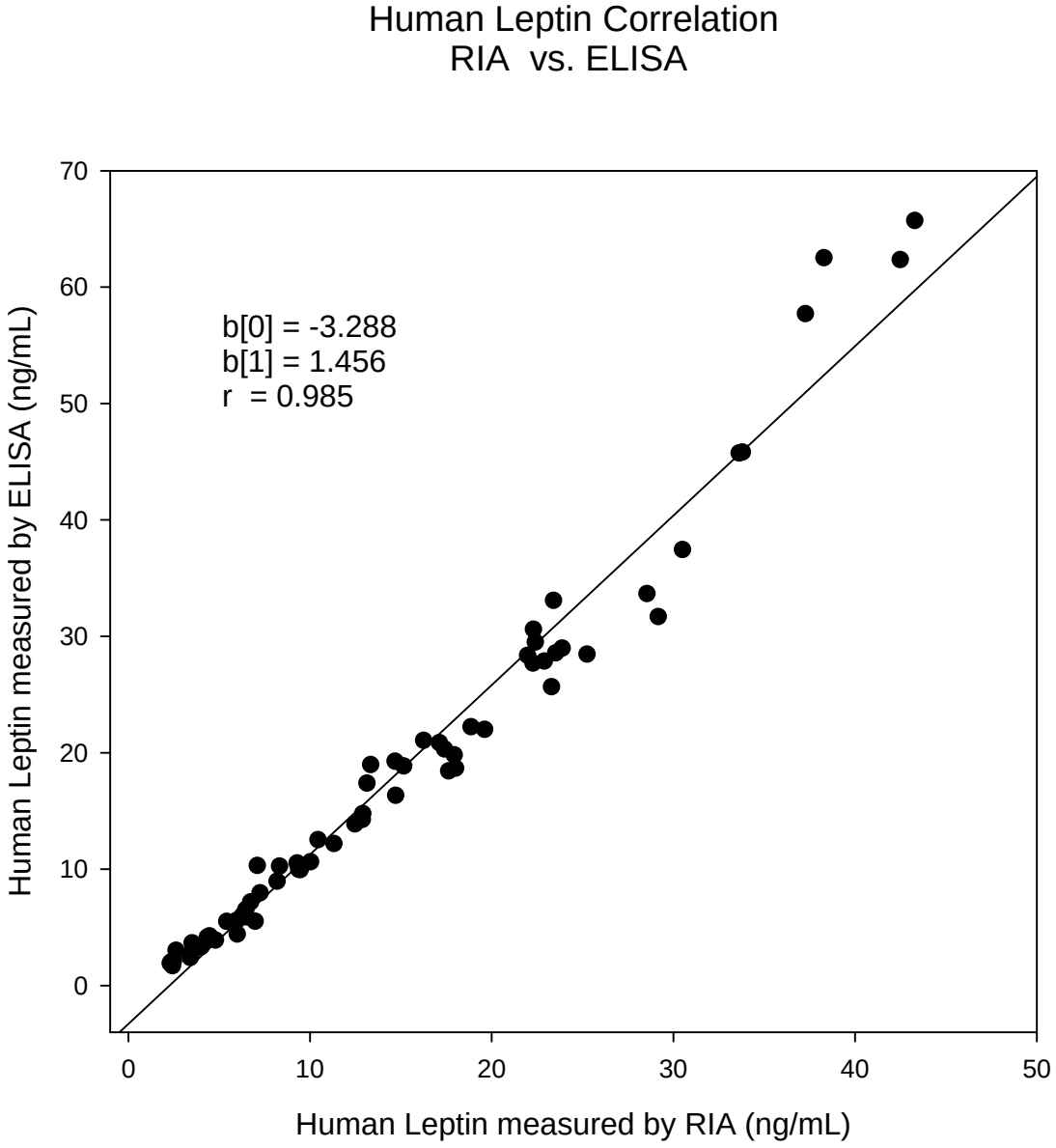
Sample No.	Volume Sampled	Expected ng/mL	Observed ng/mL	% Expected
1	25 µL	61.31	61.31	100
	12.5 µL		32.22	105.1
	5 µL		11.73	95.7
	2.5 µL		5.62	91.7
2	25 µL	33.05	33.05	100
	12.5 µL		15	90.8
	5 µL		5.55	80.4
	2.5 µL		2.65	80.2
3	25 µL	15.21	15.21	100
	12.5 µL		7.35	96.6
	5 µL		2.6	85.5
	2.5 µL		1.23	80.9

XVI. ASSAY CHARACTERISTICS (continued)

E. Linearity

Sensitive Assay				
Sample No.	Volume Sampled	Expected ng/mL	Observed ng/mL	% Expected
1	50 µL	3.50	3.50	100
	25 µL		1.88	107.3
	12.5 µL		1.00	114.4
	6.25 µL		0.51	117.2
	3.125 µL		0.24	108.3
	1.56 µL		0.12	106.9
2	50 µL	5.08	5.08	100
	25 µL		2.70	106.1
	12.5 µL		1.40	109.9
	6.25 µL		0.78	122.1
	3.125 µL		0.39	122.9
	1.56 µL		0.16	100.8
3	50 µL	6.54	6.54	100
	25 µL		3.15	96.3
	12.5 µL		1.73	105.6
	6.25 µL		0.97	118.0
	3.125 µL		0.47	114.5
	1.56 µL		0.26	125.3

XVII. CORRELATION GRAPH



66 serum samples collected from 66 human subjects were assayed for leptin content using both the Millipore Human Leptin RIA Kit (Cat.#HL-81K) and the Millipore Human Leptin ELISA Kit (Cat.#EZHL-80SK). Correlation of the two assay kits derived by linear regression analysis of paired results from each sample.

XVIII. 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/bmia.

XIX. 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 practices.
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 that 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 well cross contamination due to inappropriate mixing.
7. Remove any air bubbles formed in the well after the addition of substrate because bubbles interfere with spectrophotometric readings.
8. Do not let the absorbency reading of the highest standard reach 2.0 units or higher before adding the stop solution.
9. High absorbance in background or blank wells could be due to 1.) well cross contamination by standard solution or sample and 2.) inadequate washing of wells with HRP.

XX. REPLACEMENT REAGENTS

Reagents	**Cat. #**
Human Leptin ELISA Plate	EP81
10X HRP Wash Buffer Concentrate (50 mL)	EWB-HRP
Human Leptin ELISA Standards (1 mL/vial)	E8081-K
Quality Controls 1 & 2 (0.5 mL/vial)	6081-K
Assay Buffer (10 mL/vial)	EABTR
Enzyme Solution (12 mLl/vial)	EHRP-3
Human Leptin Detection Antibody (11 mL/vial)	E1081
Substrate (12mL)	ESS-TMB
Stop Solution (12 mL/vial)	ET-TMB

XXI. 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 Millipore products may be ordered by fax or phone. See Section A above for details on ordering.