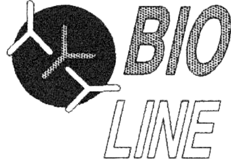

Before use, read this Package Insert.

Leptin Serum - Elisa

BL-51-E

An immunoenzymometric assay for the quantitative measurement
of human Leptin in serum and plasma.

I. INTENDED USE

An immunoenzymometric assay for the quantitative measurement of human Leptin in serum and plasma.

II. GENERAL INFORMATION

A. Name:Bio-Line **Leptin Elisa kit**
B. Catalogue number : **BL-51-E**: 96 tests
C. Manufactured by : Bio-Line S.A.
Rue André Fauchille.17 - B-1150 Bruxelles - Belgium
D. For technical assistance or ordering information contact :
Tel : +32-2-736.62.18. Fax : +32-2-742.13.15.

III. CLINICAL BACKGROUND

Leptin, the product of the *ob* gene, is a hormone secreted by adipocytes (1). Animals with mutations in the *ob* gene are obese, diabetic and have reduced activity (2). Administration of recombinant leptin to these animals decreases food intake and causes weight loss. In humans, this type of mutation has not been found.
Human leptin cDNA encodes a 167 amino acid non-glycosylated protein including a 21 AA signal peptide, which is cleaved to give mature human leptin. The human receptor for leptin (OB-R) has been identified (7) as a 1144 amino acid transmembrane glycoprotein. It is expressed in the choroid plexus and in the hypothalamus. Leptin is implicated in an increasing number of endocrine regulations including adiposity (3), satiety, energy homeostasis (4), puberty and fertility (5, 6).
Little is known about the physiologic actions of leptin in humans. The availability of a sensitive and specific assay to measure concentrations of leptin in serum and plasma will accelerate our understanding.

IV. PRINCIPLES OF THE BIO-LINE LEPTIN ELISA ASSAY

The BIO-LINE LEPTIN ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay (ELISA) performed on microtiter plate. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of human leptin.
Standards and samples react with the capture monoclonal antibody (MAb 1) coated on the microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich : coated MAb 1 - human leptin - MAb 2 - HRP, the microtiter plate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB ready for use) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiter plate is then read at the appropriate wavelength. The amount of substrate turnover is determined colorimetrically by measuring the absorbance which is proportional to the human leptin concentration. A standard curve is plotted and the human leptin concentration in a sample is determined by interpolation from the standard curve. The use of the ELISA Reader (linearity up to 3 OD units) and a sophisticated data reduction method (polychromatic data reduction) result in high sensitivity in the low range and in an extended standard range.

V REAGENTS PROVIDED

Reagents	Quantity	Colour Code	Reconstitution
Microtiter plate with 96 anti-Leptin coated wells	96 wells	blue	**Ready** for use
Standard 0 in bovine serum with preservatives	1 vial lyophilized	yellow	**Add** 1 ml distilled water
Standards 1-5 in bovine serum with preservatives (see exact value on vial labels)	5 vials lyophilized	yellow	**Add** 0.5 ml distilled water
Anti-Leptin-HRP Conjugate in a buffered solution with proteins and preservatives	11 ml	red	**Ready** for use
Controls 1 and 2 in human plasma with preservatives	2 vials lyophilized	silver	**Add** 0.5 ml distilled water
Incubation Buffer	1 vial 6 ml	black	**Ready** for use
Washing Solution (buffer with preservatives)	1 vial 10 ml	brown	**Dilute** the vial content in 2000 ml distilled water
Chromogenic Solution : TMB (Tetrametylbenzidine)	1 vial 25 ml	brown	**Ready** for use
Stop Solution	1 vial 25 ml	white	**Ready** for use

Note : Standard 0 must be used for samples dilution.

VI PRECAUTIONS AND WARNINGS

1. The human blood components included in this kit have been tested by European approved and USA FDA approved methods and found negative for HBsAg, anti-HCV and anti-HIV-1 and 2. No known method can offer complete assurance that human blood derivatives will not transmit hepatitis, AIDS or other infections.
   Therefore, handling of reagents, serum, or plasma specimens should be in accordance with local safety procedures.
2. Avoid any skin contact with Stop Solution and Chromogenic Solution (TMB ready for use). In case of contact wash thoroughly with water.
3. Do not eat, drink, smoke or apply cosmetics where kit reagents are used.
4. Do not pipet liquids by mouth.

VII EQUIPMENT AND SUPPLIES REQUIRED BUT NOT PROVIDED

1. High quality distilled water.
2. Precision pipette : 50 µl, 100 µl, 500 µl and 1 ml.
3. Vortex mixer and magnetic stirrer.
3. Horizontal microtiter plate shaker capable of 700 rpm ± 100 rpm, microtiter plate reader capable of reading at 450 nm and 490 nm, microtiter plate washer.

VIII REAGENT PREPARATION

1. **Standards and controls** : Reconstitute the lyophilized Standards and controls to the volume specified on the vial label with distilled water (1 ml for Standard 0, 0.5 ml for Standards 1-5 and Controls). Allow them to remain undisturbed until completely dissolved, then mix well by gentle inversion.
2. **Wash Solution**: Dilute the content of the Washing solution vial in 2000 ml distilled water (use a magnetic stirrer).

IX STORAGE AND SHELF LIFE OF REAGENTS

A. UNOPENED vials

Store the unopened vials at 2 C to 8 C. All kit components are stable until the expiry date printed on the labels.

B. OPENED vials

1. The anti-Leptin-HRP conjugate vial must be stored at 2 to 8 C.
2. The Standards and Controls have to be used immediately after reconstitution, and to be frozen immediately after use.
   Only one freeze/thaw cycle is possible, discard the standards and controls after the second use.
3. Store the unused strips at 2 C to 8 C in the sealed bag containing the dessicant until expiration date.
4. The Washing solution is stable at room temperature until expiration date. In order to avoid washerhead obstructions, it is recommended to prepare a fresh diluted Wash Solution each day.
5. The Chromogenic Solution (TMB ready for use) and the Stop Solution are stable at 2 C to 8 C until expiration date.

X SPECIMEN COLLECTION AND PREPARATION

* Serum samples and EDTA or heparin plasma are recommended for this assay.
* Do not use hemolyzed samples.
* Do not use lipemic samples.
* If a specimen is expected or known to have a concentration above the highest standard, it has to be diluted with the Standard 0 to fall within the measuring interval.
* If samples are not assayed the same day as the blood collection, then it is advisable to freeze them until the assay.
* Samples can only be thawn once.
* For repeat testing, freeze them in aliquots and discard each sample after first thawing.

XI BIO-LINE LEPTIN ELISA PROCEDURE

The instructions of the assay procedure must be followed to obtain reliable results.

A. Procedural notes

1. Allow the samples and reagents to equilibrate to room temperature (18 C to 25 C) before commencing the assay. Thoroughly mix the reagents and samples before use by gentle agitation or swirling.
2. Do not use kit components beyond the expiration date.
3. Do not mix materials from different kit lots.
4. Do not mix strips from different plates.
5. Perform Standards, Controls and Unknowns in duplicate. Vertical alignment is recommended.
6. A standard curve should be run with each assay run or each plate run.
7. To avoid drift, the time between pipetting of the first standard and the last sample must be no longer than 30 minutes. Otherwise, results will be affected.
8. Use a clean disposable plastic pipette for each reagent, standard, control or specimen addition in order to avoid cross contamination .
9. For the dispensing of the Chromogenic Solution and Stop Solution avoid pipettes with metal parts.
10. Use a clean plastic container to prepare the Wash Solution.
11. Dispense the Chromogenic Solution within 15 min. following the washing of the microtiter plate.
12. During incubation with Chromogenic Solution, avoid direct sunlight on the microtiter plate.
13. Respect the incubation times described in the assay procedure.

B. Assay Procedure

1. **Select the required number of strips for the run.** The unused strips should be resealed in the bag with desiccant and stored at 2-8 C.
2. **Secure** the strips into the holding frame.
3. **Pipette 50 µl of each Standard, Control, or Sample** into the appropriate wells.
4. **Pipette 100 µl of anti-Leptin Conjugate** into all the wells.
5. **Pipette 50 µl of Incubation Buffer** into all the wells.
6. **Incubate** for **2 hours** at room temperature, on an horizontal shaker set at 700 ± 100 rpm.
7. **Aspirate** the liquid from each well ;
8. **Wash** the plate four times by :
   a) dispensing of 0.4 ml of Bio-Line Wash Solution into each well ;
   b) aspirating the content of each well.
9. **Pipette 100 µl of Chromogenic Solution** into each well within 15 min. following the washing step.
10. **Incubate** the plate for **30 minutes** at room temperature on an horizontal shaker set at 700 ±100 rpm, avoiding direct sunlight.
11. **Pipette 200 µl of Stop reagent** into each well.
12. **Read** absorbances at 450 nm and 490 nm (reference filter : 630 or 650 nm) within 3 hours and calculate the results as described in section XI.

XII CALCULATION OF ANALYTICAL RESULTS

A. Reading the plate with the Bio-Line ELISA Reader

. Read the plate according to the instructions of the ELISA Reader and ELISA[AID] Software.

B. Reading the plate with other equipment

1. Polychromatic model

. Read the microtiter plate at 450 nm (reference filter : 630 or 650 nm).

. Construct a standard curve using all standard points for which absorbances are below the limit of linearity of reader used.

. Plot the OD on the ordinate against the standard concentrations on the abscissa using either linear or semi-log graph paper and draw the curve by connecting the plotted points with straight lines.

. Determine Leptin concentrations of Samples or Controls for which absorbance is no greater than those of the last standard plotted at 450 nm.

. If any Control or Sample has an absorbance greater than the absorbance of the last standard read at 450 nm, a second reading at 490 nm (reference filter: 630 or 650 nm) is needed. Proceed as described above to construct a second standard curve at 490 nm using all the standard points. The segment of the curve drawn between the last standard read at 450 nm and the most concentrate standard will be considered at 490 nm. The concentration of Samples and Controls for which absorbance is included in this segment, is read at 490 nm. So, the first reading gives the high sensitivity of the assay and the second reading allows an extended standard range.

2. Monochromatic model

. It allows to obtain results more quickly but with a loss in sensitivity (see example).

. Read the microtiter plate at 405 nm (reference filter 650 nm).

. Plot the OD on the ordinate against the standard concentrations on the abscissa using either linear or semi-log graph paper and draw the curve by connecting the plotted points with straight lines.

Note : The readings at 490 nm are only for off-scale values at 450 nm (above the limit of reader linearity)and should not replace the reading at 450 nm for values below the limit of reader linearity.

C. Example of a typical reference curve

The following data are for demonstration purpose only and can not be used in place of data generated at the time of assay. The data are provided by using the Bio-Line ELISA reader and the Bio-Line ELISA[AID] software (for the polychromatic model).

LEPTIN-ELISA		**Polychromatic model (OD)**	**Monochromatic model (OD)**
Standard	0 ng/ml	0.018	0.015
	1 ng/ml	0.109	0.043
	2 ng/ml	0.237	0.083
	10 ng/ml	1.102	0.355
	55 ng/ml	5.239	1.622
	120 ng/ml	7.358	2.291

XIII QUALITY CONTROL

- The **two Controls** provided in the kit can be used as internal laboratory controls.
  Note : Other controls which contain azide will interfere with the enzymatic reaction and cannot be used.
- Serum, EDTA plasma or heparin plasma pools can be collected and frozen immediately in aliquot to serve as controls. Repeated freezing and thawing are not permitted.
- **Record keeping** : it is good laboratory practice to record the kit lot numbers and date of reconstitution for the reagents in use.
- **Controls** : it is recommended that Controls be routinely assayed as unknown samples to measure assay variability. It is recommended that quality controls charts be maintained to monitor the performance of the kits. Control ranges are indicated on vial labels. Out of range control results indicate the assay must be repeated. Repeat patient samples may also be used to measure interassay precision.
- **Sample handling** : strictly adhere to the instruction for handling and storage of samples. Standards, Controls, and Unknowns should be run in duplicate. A clean disposable tip should always be used to avoid carryover contamination.
- **Data reduction** : it is good practice to construct a standard curve for each run to check visually the curve fit selected by the computer program.

XIV REFERENCE INTERVAL

- Normal values : range has been evaluated in healthy lean adult population.
  Lean men : 2.4 ± 1.1 ng/ml
  Lean women : 6.6 ± 3.0 ng/ml

XV PERFORMANCE CHARACTERISTICS

1. **Minimum Detectable Concentration (MDC).**
   The MDC is estimated at 0.1 ng/ml and is defined as the concentration of Leptin which corresponds to the average OD of the standard 0 ng/ml + 2 standard deviations.

2. **Precision**

INTRA-ASSAY				INTER-ASSAY (day-to-day)			
Sample	**n**	**<X> ± SD (ng/ml)**	**CV (%)**	**Sample**	**n**	**<X> ± SD (ng/ml)**	**CV (%)**
A	20	14.8 ± 0.5	3.6	A			
B	20	50.4 ± 2.6	5.2	B			

3. **Accuracy**

RECOVERY

Added Leptin (ng/ml)	**Measured Leptin (ng/ml)**	**Recovery (%)**
80	81.9	102
40	41.4	103
20	21.9	109
10	9.7	97

4. **Dilution Test**

DILUTION TEST

Dilution	**Theor. Conc. (ng/ml)**	**Meas. Conc. (ng/ml)**	**Recovery (%)**
1/1	70.6	70.6	-
1/2	35.3	37.7	107
1/4	17.6	19.1	108
1/8	8.8	9.1	103
1/16	4.4	4.6	104
1/32	2.2	2.3	104
1/64	1.1	1.1	100

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XVII SUMMARY OF ASSAY PROCEDURE

	Standards (µl)	**Controls Serum/plasma samples (µl)**
Standards (0-5) **Serum/plasma samples, controls**	50 -	- 50
Anti-Leptin-HRP Conjugate **Incubation Buffer**	100 50	100 50
Incubate for 2 hours at RT with continuous shaking (700 rpm) **Aspirate** the contents of each well **Wash** 4 times with 0.4 ml of Wash Solution and aspirate		
Chromogenic Solution (TMB)	100	100
Incubate 30 minutes at R.T. with continuous shaking (700 rpm)		
Stop Solution	200	200
Read on a microtiter plate reader and record the absorbance of each well at 450 nm (versus 630 or 650 nm) and 490 nm (versus 630 or 650 nm).		

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