

Mercodia

Leptin ELISA

Directions for Use





10-1199-01
REAGENTS FOR 96 DETERMINATIONS

For Research Use Only
Not for Use in Diagnostic Procedures

Manufactured by

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EXPLANATION OF SYMBOLS USED ON LABELS

 $\Sigma = 96$	Reagents for 96 determinations
	Expiry date
	Store between 2-8°C
	Lot No.

INTENDED USE

Mercodia Leptin ELISA provides a method for the quantitative determination of human leptin in serum and plasma.

SUMMARY AND EXPLANATION OF THE TEST

Leptin is a 16 kDa hormone secreted mainly by adipose tissue. The name of the protein is derived from the Greek word leptos meaning thin and refers to its ability to regulate energy intake and energy expenditure.

The effects of leptin were first observed by studying overweight mice with a mutation in the obese (*ob*) gene. Administration of leptin to these mice resulted in weight loss, decreased food intake and a reduction of body fat. Further research has shown that humans with high body mass index (BMI) have high levels of leptin in the blood. This observation indicates that most obese individuals are leptin resistant rather than leptin deficient.

Since the discovery of the *ob* gene product, the biological action of leptin has been broadened. Apart from its metabolic effects it has also been reported to be involved in immune function and reproduction. Moreover, leptin has been suggested to be involved in atherosclerotic disease. Studies have shown an association between leptin levels and oxidized LDL in postmenopausal women.

For clinical purposes it is important to note that leptin secretion shows a moderate circadian rhythm with a peak during the night. Serum levels are reported higher in women than in men.

PRINCIPLE OF THE PROCEDURE

Mercodia Leptin ELISA is a solid phase two-site enzyme immunoassay. It is based on the direct sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the leptin molecule. During incubation, leptin in the sample reacts with peroxidase-conjugated anti-leptin antibodies and anti-leptin antibodies bound to the microtiter well. A simple washing step removes unbound enzyme labeled antibody, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by addition of acid, giving a colorimetric endpoint that is read spectrophotometrically.

WARNINGS AND PRECAUTIONS

- For research use only. Not for use in diagnostic procedures.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- All samples should be handled as capable of transmitting infections.
- Each well can only be used once.
- The Stop Solution contains <5% Sulphuric acid.
The Stop Solution is labeled:



Danger

H318 – Causes serious eye damage.

H315 – Causes skin irritation.

P280 – Wear protective gloves. Wear eye or face protection.

P264 – Wash hands thoroughly after handling.

P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.

P332 + P313 - If skin irritation occurs: Get medical attention.

P305 + P351 + P338 + P310 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

- The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4, 5 and Wash Buffer contain <0.06% reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one. The Enzyme Conjugate Buffer, the Calibrators and Wash Buffer are labeled:



Warning

H317 – May cause an allergic skin reaction.

P280 – Wear protective gloves.

P261 – Avoid breathing vapour.

P272 – Contaminated work clothing should not be allowed out of the workplace.

P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.

P333 + P313 – If skin irritation or rash occurs: Get medical attention.

P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Tubes for sample preparation
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader with 450 nm filter
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

REAGENTS

Each Mercodia Leptin ELISA kit (10-1199-01) contains reagents for 96 wells, sufficient for 42 samples and one calibrator curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2–8°C.

Coated Plate Mouse monoclonal anti-human leptin For unused microplate strips, reseal the bag using adhesive tape, store at 2–8°C and use within 2 weeks.	1 plate	96 wells 8-well strips	Ready for use
Calibrators 1, 2, 3, 4, 5 Recombinant human leptin Color coded yellow Concentration stated on vial label Storage after reconstitution: 2–8°C for 2 months. For storage of reconstituted Calibrators for more than 2 months., store at - 20°C.	5 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial.
Calibrator 0 Color coded yellow	1 vial	1000 µL	Ready for use
Sample Buffer Color coded yellow	1 bottle	50 mL	Ready for use
Enzyme Conjugate 11X Peroxidase conjugated mouse monoclonal anti-human leptin	1 vial	1.3 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue	1 vial	13 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2–8°C for 2 months.	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution

Substrate TMB Colorless solution <i>Note! Light sensitive!</i>	1 bottle	22 mL	Ready for use
Stop Solution 0.5 M H ₂ SO ₄	1 vial	7 mL	Ready for use

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer according to the table below.

When preparing enzyme conjugate 1X solution for the whole plate or if the reagents are to be used within 2 months, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial. Mix gently.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	0.7 mL	7 mL
4 strips	0.4 mL	4 mL

Storage after dilution: 2-8°C for 2 months.

SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow to clot, and separate the serum by centrifugation. Specimen can be stored at 2-8°C up to 2 weeks. For longer periods, store samples at -20°C. Avoid repeated freezing and thawing.

Plasma

Collect blood by venipuncture into tubes containing heparin, citrate or EDTA as anticoagulant, and separate the plasma fraction by centrifugation. Samples can be stored at 2-8°C up to 2 weeks. For longer periods store samples at -20°C. Avoid repeated freezing and thawing.

PREPARATION OF SAMPLES

The measuring range is adjusted for sample dilution 1/11, e.g. 25 µL + 250 µL Sample Buffer. However, samples below Calibrator 1 should be run undiluted and samples above Calibrator 5 should be diluted 1/101 e.g. 25 µL + 2500 µL Sample Buffer.

Note! Buffers containing sodium azide (NaN₃) cannot be used for sample dilution.

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Prepare a calibrator curve for each assay run and plate. The product has been optimized and validated without plate sealer.

1. Prepare enzyme conjugate 1X solution, wash buffer 1X solution, controls and samples.
2. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
3. Pipette 25 μ L each of Calibrators, controls and samples into appropriate wells.
4. Add 100 μ L of enzyme conjugate 1X solution into each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours at room temperature (18-25°C).
6. Wash 6 times with 700 μ L wash buffer 1X solution per well using an automatic plate washer with overflow-wash function, after final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure. Or manually, discard the reaction volume by inverting the microplate over a sink. Add 350 μ L wash buffer 1X solution to each well. Discard the wash solution, tap firmly several times against absorbent paper to remove excess liquid. Repeat 5 times. Avoid prolonged soaking during washing procedure.
7. Add 200 μ L Substrate TMB into each well.
8. Incubate for 15 minutes on the bench at room temperature (18-25°C).
9. Add 50 μ L Stop Solution to each well. Place the plate on the shaker for approximately 5 seconds to ensure mixing.
10. Read optical density at 450 nm and calculate results. Read within 30 minutes.

Note! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution.

INTERNAL QUALITY CONTROL

Commercial control such as Mercodia Obesity Control (10-1241-01) and/or internal serum pools with low, intermediate and high leptin concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number, preparation dates of kit components, OD values for the blank, Calibrators and concentrations of controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

CALCULATION OF RESULTS

Computerized calculation

The concentration of leptin is obtained by computerized data reduction of the absorbance for the Calibrators, except for Calibrator 0, versus the concentration using four parameter logistic or cubic spline regression.

Manual calculation

1. Plot the absorbance values obtained for the Calibrators, except Calibrator 0, against the leptin concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the samples from the calibrator curve.
3. Multiply the concentration with the dilution factor.

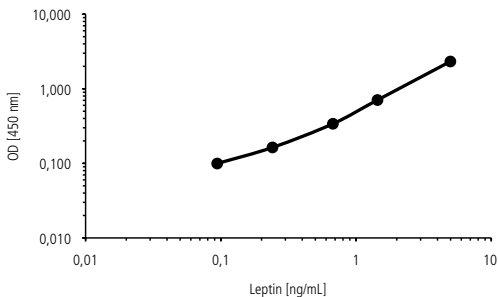
Example of results

Wells	Identity	A ₄₅₀	Mean conc.	x11 ng/mL
1A–B	Calibrator 0	0.057/0.058		
1C–D	Calibrator 1*	0.094/0.105		
1E–F	Calibrator 2*	0.162/0.165		
1G–H	Calibrator 3*	0.337/0.342		
2A–B	Calibrator 4*	0.695/0.719		
2C–D	Calibrator 5*	2.161/2.485		
2E–F	Sample 1	0.166/0.164	0.254	2.79
2G–H	Sample 2	0.304/0.297	0.562	6.18
3A–B	Sample 3	0.876/0.898	1.923	21.2

*Concentration stated on vial label.

Example of calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



LIMITATIONS OF THE PROCEDURE

Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

EXPECTED VALUES

Good practice dictates that each laboratory establishes its own expected range of values.

PERFORMANCE CHARACTERISTICS

Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured.

The detection limit is 0.024 ng/mL as determined by methodology described in ISO11843-Part 4.

Concentration of samples with absorbance below Calibrator 1 should not be calculated, instead expressed as less or equal to (\leq) the concentration indicated on the vial for Calibrator 1.

Recovery

Recovery upon addition is 88-104% (mean 93%).

Recovery upon dilution is 89-115% (mean 102%).

Hook effect

Samples with a leptin concentration of up to 100 000 ng/mL can be measured without giving falsely low results.

Precision

Each sample was analyzed in 4 replicates on 17 different occasions.

Sample	Mean value ng/mL	Coefficient of variation	
		Repeatability %*	Within laboratory %**
1	0.26	3.1	8.5
2	0.54	2.4	7.1
3	1.75	1.8	5.2

*Within assay variation

**Total assay variation

Specificity

The following cross reactions have been found:

CNTF	n.d.
G-CSF	n.d.
IL-6	n.d.
IL-11	n.d.
IL-12	n.d.
LIF	n.d.
Oncostatin M	n.d.
Rat Leptin	0.02%
Mouse Leptin	n.d.
Sheep Leptin	0.003%

n.d.=not detected

The soluble leptin receptor gives a 50 % inhibition of the measured leptin level at receptor concentrations between 30-50 ng/mL.

CALIBRATION

Mercodia Leptin ELISA is calibrated against an in-house reference preparation of Leptin.

WARRANTY

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorized distributors, in such event, shall not be liable for damages indirect of consequential.

REFERENCES

Chan DC, Watts GF, Ng TWK, Uchida Y, Sakai N, Yamashita S and Barrett PHR (2005) Adiponectin and other Adipocytokines as Predictors of Markers of Triglyceride-Rich Lipoprotein Metabolism. *Clin Chem* 51:578-585.

Friedman JM and Halaas JL (1998) Leptin and the Regulation of Body Weight in Mammals. *Nature* 395:763-770.

Havel PJ (2002) Control of Energy Homeostasis and Insulin Action by Adipocyte Hormones: Leptin, Acylation Stimulating Protein and Adiponectin. *Current Opinion in Lipidology* 13:51-59.

Meier U and Gressner AM (2004) Endocrine Regulation of Energy Metabolism Review of Pathobiochemical and Clinical Aspects of Leptin, Ghrelin, Adiponectin, and Resistin. *Clin Chem* 50:1511-1525.

Porreca E, DiFebbo C, Moretta V, Angelini A, Guglielmi MD, DiNisio M and Cuccurullo F (2004) Circulating Leptin Is Associated with Oxidized LDL in Postmenopausal Women. *Atherosclerosis* 175:139-143.

Verdich C, Toubro S, Buemann B, Holst JJ, Bülow J, Simonsen L, Søndergaard SB, Christensen NJ and Astrup A (2001) Leptin Levels Are Associated with Fat Oxidation and Dietary-Induced Weight Loss in Obesity. *Obesity Research* 9:452-461.

Zhang F, Chen Y, Heiman M and DiMarchi R (2005) Leptin: Structure, Function and Biology. *Vitamins and Hormones* 71:345-372.

Further references can be found on our website: www.mercodia.com

SUMMARY OF PROTOCOL SHEET

Merckodia Leptin ELISA

Add Calibrators, controls* and samples	25 μ L
Add enzyme conjugate 1X solution	100 μ L
Incubate	2 hours at 18-25°C on a plate shaker (700-900 rpm)
Wash plate with wash buffer 1X solution	700 μ L, 6 times
Add Substrate TMB	200 μ L
Incubate	15 minutes at 18-25°C
Add Stop Solution	50 μ L Shake for 5 seconds to ensure mixing
Measure A_{450}	Evaluate results

*not included

For full details see page 7

For technical support please contact: support@merckodia.com