

Merckodia 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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Merckodia 

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 16kDa 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. Not for internal or external use in humans or animals.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animal or swine.
- The Stop Solution in this kit contains 0.5 M H₂SO₄. Follow routine precautions for handling hazardous chemicals.
- All patient samples should be handled as capable of transmitting infections.

MATERIAL REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100, 200 μ l (repeating pipettes preferred for addition of enzyme conjugate solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Microplate reader (450 nm filter)
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device

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)	1 plate 8-well strips	96 wells	Ready for use
For unused microtitration strips, reseal the bag with adhesive tape, store at 2-8°C and use within 2 months.			
Calibrators 1, 2, 3, 4, 5 (Recombinant human leptin) Concentration indicated on vial label. Color coded yellow	5 vials	1000 µl	Ready for Use
Calibrator 0 Color coded yellow	1 vial	1000 µl	Ready for use
Sample Buffer Color coded yellow	1 vial	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 two months	1 bottle	40 ml	Dilute with 800 ml redistilled water to make wash buffer.
Substrate TMB (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 solution

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

When preparing enzyme conjugate 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
6 strips	0.5 ml	5 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. Separate the serum by centrifugation at 4300g for 15 minutes at 2-8°C. Specimen can be stored at 2-8°C up to 14 days. 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. Samples can be stored at 2-8°C up to 14 days. 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.

1. Prepare enzyme conjugate solution, wash buffer and samples.
2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
3. Pipette 25 μ l each of Calibrators and samples into appropriate wells.
4. Add 100 μ l of enzyme conjugate solution into each well.
5. Incubate on a plate shaker (700-900 rpm) for 2 hours at room temperature (18-25°C).
6. Wash plate 6 times with 350 μ l per well.*
After final wash, invert and tap the plate firmly against absorbent paper.
7. Add 200 μ l Substrate TMB into each well.
8. Incubate for 15 minutes 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.

* The plate can be washed with an automatic washer or manually. When washing with an automatic washer the overflow function is an advantage if available. Manual wash can be done either with a pipette or a squirt bottle: Discard the reaction volume by inverting the microplate over a sink and shake to remove the liquid. Add wash buffer to each well with a pipette or fill the wells completely by spraying wash buffer into wells with a squirt bottle. Invert the microplate over a sink and shake to remove the wash buffer. Repeat five times.

Note! To prevent contamination between the conjugate and substrate, separate pipettes are recommended.

INTERNAL QUALITY CONTROL

Commercial control and/or internal serum pools with low, intermediate and high leptin concentrations should routinely be assayed as unknowns, 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.

CALCULATION OF RESULTS

Computerized calculation

The concentration of leptin is obtained by computerized data reduction of the absorbance for the Calibrators, except Calibrator 0, versus the concentration using 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 unknown samples from the calibrator curve.
3. Multiply the concentration with the dilution factor.

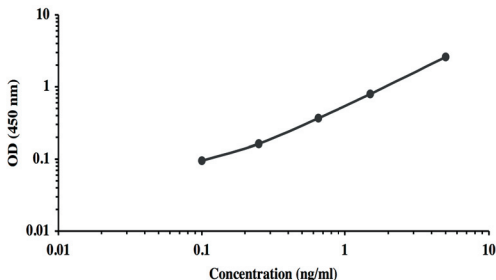
Example of results

Wells	Identity	A_{450}	Mean conc. ng/ml	x11 ng/ml
1A-B	Calibrator 0	0.060/0.061		
1C-D	Calibrator 1 (0.10 ng/ml)*	0.096/0.095		
1E-F	Calibrator 2 (0.25 ng/ml)*	0.161/0.166		
1G-H	Calibrator 3 (0.65 ng/ml)*	0.363/0.372		
2A-B	Calibrator 4 (1.5 ng/ml)*	0.778/0.820		
2C-D	Calibrator 5 (5.0 ng/ml)*	2.573/2.650		
2E-F	Unknown 1	0.158/0.165	0.242	2.66
2G-H	Unknown 2	0.310/0.322	0.550	6.05
3A-B	Unknown 3	0.957/0.993	1.815	20.0

*Exact concentration indicated 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

As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated. 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 0.05 (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 91-109% (mean 96%).

Recovery upon dilution is 92-114% (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 24 different occasions.

Sample	Mean value (ng/ml)	Coefficient of variation		
		within assay %	between assay %	total assay %
1	0.25	2.8	4.5	4.8
2	0.55	3.0	4.6	4.8
3	1.88	2.3	5.2	5.3

Specificity

The following cross reactions have been found:

CNTF	≤ 0.05%
G-CSF	≤ 0.0004%
IL-6	≤ 0.0002%
IL-11	≤ 0.0008%
IL-12	≤ 0.0004%
LIF	≤ 0.002%
Oncostatin M	≤ 0.0002%
Rat Leptin	0.02 %
Mouse Leptin	≤ 0.001 %
Sheep Leptin	0.003%

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 the rDNA-derived Human Leptin 1st International Standard (IS) NIBSC Code 97/594, produced by the WHO International Laboratory for Biological Standards at NIBSC.

WARRANTY

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

SUMMARY OF PROTOCOL SHEET

Add Calibrators, controls and samples	25 μ l
Add enzyme conjugate solution	100 μ l
Incubate	2 hour at 18-25°C on a plate shaker
Wash plate with wash buffer	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 A450	Evaluate results