AccuDiag™ Leptin ELISA Kit

REF 1742-6

IVD See External Label 2°C 8°C 96 Tests

Leptin ELISA	
Method	Enzyme Linked Immunosorbent Assay
Principle	ELISA: Solid phase enzyme linked immunosorbent assay
Detection Range	0.7-100 ng/mL
Sample	15 µL serum
Sensitivity	0.7 ng/mL
Incubation Time	195 minutes
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The **DAI Leptin Enzyme** is an enzyme immunoassay for the quantitative determination of Leptin in serum and Li- Heparin plasma.
This assay is intended for in vitro diagnostic use and Laboratory Professional Use only.

SIGNIFICANCE AND SUMMARY

Leptin is produced primarily in the adipocytes of white adipose tissue and circulates in blood in free form and bound to proteins (1). In mammals, leptin is pleiotropic, regulating a multitude of physiological processes. Leptin reduces appetite and food intake, and inhibits hepatic glucose production, fatty acid synthesis and the expression of resistin. In contrast, Leptin increases energy expenditure by inducing oxidation of fatty acids in liver and muscle. Moreover, Leptin stimulates insulin secretion and glucose uptake as well as secretion of inflammatory cytokines (2,3). Leptin serves as a lipostatic signal and conveys critical information regarding metabolic state to the brain by stimulating anorexic proopiomelanocortin/cocaine and amphetamine-related transcript neurons and inhibiting orexigenic neuropeptide Y/ agouti-related protein neurons (4,5). The actions of Leptin are opposed by the hormone ghrelin. Both hormones act on receptors in the arcuate nucleus of the hypothalamus to regulate appetite to achieve energy homeostasis (6).

Although leptin reduces appetite as a circulating signal, obese individuals generally exhibit a higher circulating concentration of leptin than normal weight individuals due to their higher percentage body fat (7). These people show resistance to leptin, similar to resistance of insulin in type 2 diabetes, with the elevated levels failing to control hunger and modulate their weight. In obesity, a decreased sensitivity to leptin occurs, resulting in an inability to detect satiety despite high energy stores (8). Although regulation of fat stores is deemed to be the primary function of leptin, it also plays a role in other physiological processes, as evidenced by its multiple sites of synthesis other than fat cells, and the multiple cell types beside hypothalamic cells that have leptin receptors.
Leptin-deficient pathologies are typically accompanied by hyperphagia and obesity (9,10). Extreme obesity can be observed with mutations in the leptin receptor. The anorexigenic properties of leptin have been well characterized in the context of leptin-deficient humans, resulting in the reduction of food intake and body mass (11,12).
In conclusion, Leptin can be measured for the differential diagnosis of obesity with leptin resistance.

ASSAY PRINCIPLE

The DAI Leptin ELISA Kit is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a Leptin molecule.
During the first incubation, leptin in the added sample binds to the immobilized Antibody.
Unbound material is washed off.
In the second incubation, added Antiserum, which contains biotinylated anti-leptin Antibody forms a sandwich complex with the bound leptin on the microtiter wells.
After incubation the unbound material is washed off and a Steptavidin Peroxidase Enzyme Complex is added for detection of the bound Leptin molecules.
After a washing step to remove all unbound substances, the solid phase is incubated with the substrate solution. The colorimetric reaction is stopped by the addition of stop solution, and Optical Density (OD) of the resulting yellow product is measured. The intensity of the color is propotional to the concentration of analyte in the sample.
A standard curve is constructed by plotting OD values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

SPECIMEN COLLECTION AND PREPARATION

Serum or plasma can be used in this assay. Do not use haemolytic, icteric or lipaemic specimens. Please note: Samples containing sodium azide should not be used in the assay.

1. **Specimen Collection**

Serum: Collect blood by venipuncture (e.g. Sarstedt Monovette for serum), allow to clot, and separate serum by centrifugation at room temperature. Do

not centrifuge before complete clotting has occurred. Patients receiving anticoagulant therapy may require increased clotting time.

Plasma:
Whole blood should be collected into centrifuge tubes containing anti-coagulant (e.g. Sarstedt Monovette with the appropriate plasma preparation) and centrifuged immediately after collection.

2. Specimen Storage
Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying.
Specimens held for a longer time should be frozen only once at -20°C prior to assay. Thawed samples should be inverted several times prior to testing.

3. Specimen Dilution
If in an initial assay, a specimen is found to contain more than the highest standard, the specimens can be diluted with *Standard 0* and reassayed as described in Assay Procedure.
For the calculation of the concentrations this dilution factor has to be taken into account.
Example:
a) dilution 1:10: 10 µl Serum + 90 µl Standard 0 (mix thoroughly)
b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Standard 0 (mix thoroughly)

REAGENTS

Materials provided with the test kit

1. **Microtiter wells**, 12x8 (break apart) strips, 96 wells; Wells coated with anti-Leptin antibody (monoclonal).
2. **Standard (Standard 0-5),** 6 vials, 0.5ml, (Lyophilized); Concentrations: 0, 2, 5, 25, 50 and 100 ng/ml.
   The standards are calibrated against the following reference material: WHO International Standard Leptin, human NIBSC Code 97/594
   See "Reagent Preparation". Contain non mercury preservative.
3. **Control (Low and High)**, 2 vials, 0.5ml (Lyophilized) For control values and ranges please refer to vial label or QC-Datasheet. "See Preparation of Reagents" Contain non-mercury preservative.
4. **Assay Buffer**, 1 vial, 11 ml, ready to use, Contain non-mercury preservative.
5. **Antiserum**, 1 vial, 11 ml, ready to use, monoclonal biotinylated anti-Leptin antibody; Contain non-mercury preservative.
6. **Enzyme Complex**, 1 vial, 11 ml, ready to use, streptavidin conjugated to horseradish Peroxidase; Contain non-mercury preservative.
7. **Substrate Solution**, 1 vial, 14 ml, ready to use, Tetramethylbenzidine TMB.
8. **Stop Solution**, 1 vial, 14 ml, ready to use, contains 0.5M H_2SO_4, Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **Wash Solution**, 1 vial, 30 ml (40X concentrated), see "Reagents Preparation".

Materials required but not provided

1. A microtiter plate standard reader (450±10 nm) (e.g. the DAI Microtiter Plate Reader)
2. Calibrated variable precision micropipettes
3. Absorbent paper
4. Distilled or deionized water
5. Timer
6. Graph paper or software for data reduction

STORAGE

When stored at 2°C - 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
Opened reagents must be stored at 2°C - 8°C. Microtiter wells must be stored at 2°C - 8°C. Once the foil bag has been opened, care should be taken to close it tightly again.

REAGENT PREPARATION

Bring all reagents and required number of strips to reach room temperature prior to use.

Standards
Reconstitute the lyophilized contents of each vial with 0.5 mL deionized water and let stand for at least 10 minutes at room temperature. Mix several times before use.
Note: The reconstituted standards are stable for at least 6 weeks at 2-8°C. For longer storage freeze at -20°C.

Controls
Reconstitute the lyophilized content of each vial with 0.5 ml deionized water and let stand for at least 10 minutes at room temperature. Mix the control several times before use.
Note: The reconstituted control is stable for at least 6 weeks at 2-8°C. For longer storage freeze at -20°C.

Wash Solution
Add deionized water to the 40X concentrated Wash Solution.
Dilute 30 ml of concentrated Wash Solution with 1170 mL deionized water to a final volume of 1200 ml.
The diluted Wash Solution is stable for 2 weeks at room temperature.

Disposal of the Kit
The disposal of the kit must be made according to the national regulations. Special information for this product is given in the Material Safety Data Sheets (see chapter 13).

Damaged Test Kits
In case of any severe damage of the test kit or components, DAI have to be informed in writing, at the latest, one week after receiving the kit. Severely damaged single components should not be used for a test run. They have to be stored until a final solution has been found. After this, they should be disposed according to the official regulations.

WARNINGS AND PRECAUTIONS

1. This kit is for in vitro diagnostic use only. For professional use only.
2. All reagents of this test kit which contain human serum or plasma have been tested and confirmed negative for HIV I/II, HBsAg and HCV by FDA approved procedures. All reagents, however, should be treated as potential biohazards in use and for disposal.
3. Before starting the assay, read the instructions completely and carefully. Use the valid version of instructions for use provided with the kit. Be sure that everything is understood.
4. The microplate contains snap-off strips. Unused wells must be stored at 2°C – 8°C in the sealed foil pouch and used in the frame provided.
5. Pipetting of samples and reagents must be done as quickly as possible and in the same sequence for each step.
6. Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may

turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.

7. Mix the contents of the microplate wells thoroughly to ensure good test results. Do not reuse microwells.
8. Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
9. Allow the reagents to reach room temperature (21°C - 26°C) before starting the test. Temperature will affect the absorbance readings of the assay. However, values for the patient samples will not be affected.
10. Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
11. Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
12. Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
13. Handling should be done in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.
14. Do not use reagents beyond expiry date as shown on the kit labels.
15. All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes and microtiter plate readers.
16. Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even of the same lot. The kits may have been shipped or stored under different conditions and the binding characteristics of the plates may result slightly different.
17. Avoid contact with Stop Solution containing 0.5 M H_2SO_4. It may cause skin irritation and burns.
18. Some reagents contain Proclin 300, BND and/or MIT as preservatives. In case of contact with eyes or skin, flush immediately with water.
19. TMB substrate has an irritant effect on skin and mucosa. In case of possible contact, wash eyes with an abundantmvolume of water and skin with soap and abundant water. Wash contaminated objects before reusing them. If inhaled, take the person to open air.
20. Chemicals and prepared or used reagents have to be treated as hazardous waste according to the national biohazard safety guideline or regulation.
21. For information on hazardous substances included in the kit please refer to Safety Data Sheets. Safety Data Sheets for this product are available upon request directly from DAI.

TEST PROCEDURE

Procedural Notes

- All reagents and specimens must be allowed to come to room temperature before use.
- All reagents must be mixed without foaming.
- Do not interchange caps of reagent vials to avoid cross contamination.
- Once the test has been started, all steps should be completed without interruption in the same sequence for each step.
- Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination.
- Mix the contents of the microtitre plate wells thoroughly t ensure good test results.
- Optical Density is a function of the incubation time and temperature. Respect the Incubation times and temperatures as given in chapter 'Test Procedure'.
- Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- The enzymatic reaction is linearly proportional to time and temperature.

Important notes to washing procedure:
Washing is critical. Improperly washed wells will give erroneous results. The sensitivity and precision of this assay is markedly influenzed by the correct performance of washing procedure.

Test Performance Using Fully Automated Analysis devices:
Automated test performance using fully automated, open-system analysis devices is possible. However the combination must be validated by the user.

ASSAY PROCEDURE

Each run must include a standard curve.
The controls serve as internal controls for the reliability of the test procedure. They must be assayed with each test run. The given test procedure describes manual processing.

1. Secure the desired number of Microtiter wells in the frame holder.
2. Dispense **15 µL** of each **Standard, Control and samples** with new disposable tips into appropriate wells.
3. Dispense **100 µL Assay Buffer** into each well.
   Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
4. Incubate for **120 minutes** at room temperature.
5. Wash the wells as follows
   If the wash step is performed manually:
   Briskly shake out the contents of the wells.
   Rinse the wells **3 times** with **300 µL** diluted Wash Solution per well.

   If an automated Platewasher is used:
   Rinse the wells **3 times** with **400 µL** diluted Wash Solution per well.

   At the end of washing step, always strike the wells sharply on absorbent paper to remove residual droplets.
6. Dispense **100 µL Antiserum** to each well.
7. Incubate for **30 minutes** at room temperature.
8. Wash as described in step 5.
9. Dispense **100 µL Enzyme Complex** into each well.
10. Incubate for **30 minutes** at room temperature.
11. Wash as described in step 5.
12. Add **100 µL** of **Substrate Solution** to each well.
13. Incubate for 15 minutes at room temperature.
14. Stop the enzymatic reaction by adding **50 µL** of **Stop Solution** to each well.
15. Measure the Optical Density of the solution in each well at **450 nm (reading) and at 620nm to 630 nm (background substraction recommended)** with a microtiter plate reader.
16. It is recommended that the wells be read **within 10 minutes** after adding the Stop Solution.

RESULTS

1. Calculate the average Optical Density (OD) values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean OD obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean OD value for each sample determine the corresponding concentration from the standard curve.
4. **Automated method:** The results in the Instructions for Use have been calculated automatically using a 4-Parameter curve fit. (4 Parameter

Rodbard or 4 Parameter Marquardt are the preferred methods.) Other data reduction functions may give slightly different results.

5. The concentration of the samples can be read directly from this standard curve. Samples with concentrations higher than that of the highest standard have to be further diluted or reported as > 100 ng/mL. For the calculation of the concentrations this dilution factor must be considered.
6. For duplicate determinations, the mean of the two values must be taken. If the two values deviate substantially from one another, we recommends retesting the samples.

Example of Typical Standard Curve

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.

Standard	Optical Units (450 nm)
Standard 0 (0 ng/ml)	0.02
Standard 1 (2 ng/ml)	0.07
Standard 2 (5 ng/ml)	0.16
Standard 3 (25 ng/ml)	0.74
Standard 4 (50 ng /ml)	1.41
Standard 5 (100 ng/ml)	2.30

EXPECTED NORMAL VALUES

It is strongly recommended that each laboratory should determine its own normal and abnormal values.
In a study conducted with apparently normal healthy adults, using the DAI Leptin ELISA the following values are observed:

Population	Range (ng/ml)
Adult with BMI (18.5 -24.9)	**<0.7 – 9.1**
Adult with BMI (25-30)	**1.3-21.2**
Adult with BMI >30	**3.4 – 32.1**
Children (1-10 years)	**<0.7 – 11.7**

The results alone should not be the only reason for any therapeutic consequences. The results should be correlated to other clinical observations and diagnostic tests.

QUALITY CONTROL

Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid.

In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.

After checking the above mentioned items without finding any error contact your distributor or DAI directly.

PERFORMANCE CHARACTERISTICS

Assay Dynamic Range

The range of the assay is between 0.7 – 100 ng/mL.

Specificity of Antibodies (Cross Reactivity)

The following substances were tested for cross reactivity of the assay:

Component	Cross reactivity
Human Leptin	100 %
Rat Leptin	<0.2%
Mouse Leptin	<0.2%
Human Insulin	N.D.
Human Proinsulin	N.D.
Rat Insulin	N.D.
Human C-Peptide	N.D.
Glucagon	N.D.
IGF-1	N.D.

N.D.: Not detectable

Sensitivity

The analytical sensitivity of the DAI ELISA was calculated by adding 2 standard deviations to the mean of 20 replicate analyses of the *Standard 0* and was found to be 0.7 ng/mL.

Reproducibility

Intra Assay

The within assay variability is shown below:

Sample	N	Mean (ng/mL)	CV (%)
1	20	1.1	9.6
2	20	3.2	7.8
3	20	27.4	8.6

Inter Assay

The between assay variability is shown below:

Sample	N	Mean (ng/mL)	CV (%)
1	6	1.4	6.9
2	6	3.7	3.7
3	6	9.7	9.1

Inter Lot

The inter-assay between the lots is shown below.

Sample	N	Mean (ng/mL)	CV (%)
1	4	6.7	11.0
2	4	20.3	11.6
3	4	1.3	7.6
4	4	14.4	8.7

Recovery

Samples have been spiked by adding Leptin solutions with known concentrations.
The % recovery has been calculated by multiplication of the ratio of the measurements and the expected values with 100 (expected value =

(endogenous Leptin + added Leptin) / 2; because of a 1:2 dilution of serum with spike material).

		Sample 1	Sample 2	Sample 3
Concentration		4.6	21.4	9.6
Average Recovery		88.8	97.0	94.8
Range of Recovery (%)	From	86.8	90.6	84.0
	To	93.1	102.1	106.0

Linearity

		Sample 1	Sample 2	Sample 3
Concentration		4.6	21.4	9.6
Average Recovery		93.2	92.7	104.7
Range of Recovery (%)	From	85.1	86.2	88.0
	To	107.5	103.1	114.3

LIMITATIONS OF PROCEDURE

Reliable and reproducible results will be obtained when the assay procedure is performed with a complete understanding of the package insert instruction and with adherence to good laboratory practice. Any improper handling of samples or modification of this test might influence the results.

Interfering Substances
Haemoglobin (up to 4 mg/mL), Bilirubin (up to 0.5 mg/mL) and Triglyceride (up to 30 mg/mL) have no influence on the assay results.
A biotin concentration of upto 1200 ng/ml in the sample has no influenze on the assay results.

Drug Interferences
Until today no substances (drugs) are known to us, which have an influence to the measurement of Leptin in a sample.

High-Dose-Hook Effect
Hook effect was not observed in this test up to a concentration of 5000 ng/mL of Leptin.

LEGAL ASPECTS

1. Reliability of Results
The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.

The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact DAI.

Therapeutic Consequences
Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.

Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.

The test result itself should never be the sole determinant for delivery any therapeutic consequences.

2. Liability
Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to Therapeutic Consequences are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
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EC REP	CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands. www.cepartner4u.eu
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