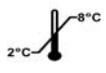
LDN®
IMMUNOASSAYS AND SERVICES
BIOGENIC AMINES & NEUROSCIENCE | ENDOCRINOLOGY | FOOD SAFETY
LABOR DIAGNOSTIKA NORD GmbH & Co.KG | Am Eichenhain 1 | 48531 Nordhorn | Germany | Tel. +49 5921 8197-0 | Fax +49 5921 8197-222 | info@ldn.de | www.ldn.de

## Instructions for use

# Leptin ELISA

REF **ME E-0300R**





RUO

For research use only – Not for use in diagnostic procedures

**Leptin ELISA**

***INTENDED USE***

For the quantitative determination of Leptin in human serum by an enzyme immunoassay method.

***PRINCIPLE OF THE TEST***

The principle of the following enzyme immunoassay test follows a typical two-step capture or 'sandwich' type assay. The assay makes use of two highly specific monoclonal antibodies: A monoclonal antibody specific for leptin is immobilized onto the microplate and another monoclonal antibody specific for a different epitope of leptin is conjugated to biotin. During the first step, leptin present in the samples and standards is bound to the immobilized antibody and to the biotinylated antibody, thus forming a sandwich complex. Excess and unbound biotinylated antibody is removed by a washing step. In the second step, streptavidin-HRP is added, which binds specifically to any bound biotinylated antibody. Again, unbound streptavidin-HRP is removed by a washing step. Next, the enzyme substrate is added (TMB), forming a blue coloured product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microtiter plate reader at 450 nm. A set of standards is used to plot a standard curve from which the amount of leptin in samples and controls can be directly read.

***PROCEDURAL CAUTIONS AND WARNINGS***

1. This kit is intended for in vitro use only.
2. Practice the following good laboratory practices when handling kit reagents:
   - Do not pipette by mouth.
   - Do not smoke, drink, or eat in areas where specimens or kit reagents are being handled.
   - Wear protective clothing and disposable gloves when handling the specimens and kit reagents.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses; in case of contact, flush with water immediately and contact a doctor.
3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Avoid microbial contamination of reagents.
5. A standard curve must be established for every run.
6. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
7. The controls (included in kit) should be included in every run and fall within established confi dence limits.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and specimens should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and specimens.
10. Improper procedural techniques, imprecise pipetting, incomplete washing as well as improper reagent storage may be indicated when assay values for the control do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
12. The substrate solution (TMB) is sensitive to light and should remain colourless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
13. When dispensing the substrate and stopping solution, do not use pipettes in which these liquids will come into contact with any metal parts.
14. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, standard and control.
15. Do not mix various lot numbers of kit components within a test and do not use any component beyond the expiration date printed on the label.
16. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.

***LIMITATIONS***

1. All the reagents within the kit are calibrated for the direct determination of leptin in human serum. The kit is not calibrated for the determination of leptin in saliva, plasma or other specimens of human or animal origin.
2. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
3. Any samples or control sera containing azide or thimerosal are not compatible with this kit, as they may lead to false results.
4. Only assay buffer may be used to dilute any high serum samples. The use of any other reagent may lead to false results.

***SAFETY CAUTIONS AND WARNINGS***

***POTENTIAL BIOHAZARDOUS MATERIAL***

All serum samples should be considered a potential biohazard and handled with the appropriate precautions.

***CHEMICAL HAZARDS***
Avoid contact with reagents containing TMB, hydrogen peroxide and sulfuric acid. If contacted with any of these reagents, wash with plenty of water. TMB is a suspected carcinogen.

***SPECIMEN COLLECTION AND STORAGE***
Approximately 0.1 ml of serum is required per duplicate determination. Collect 4–5 ml of blood into an appropriately labelled tube and allow it to clot. Centrifuge and carefully remove the serum layer. Store at 4°C for up to 24 hours or at -10°C or lower if the analyses are to be done at a later date. Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

***REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED***
- Precision pipette to deliver 20-100 µl
- Disposable pipette tips
- Distilled or deionized water
- Plate shaker
- Microplate washer (recommended)
- Microplate reader with a filter set at 450 nm and an upper OD limit of 3.0 or greater

***REAGENTS PROVIDED***

**1. AA E-0030** WASH-CONC 10x **Wash Buffer Concentrate –** Requires Preparation **X10**

| | |
|---|---|
| Contents: | One bottle containing buffer with a non-ionic detergent and a non-mercury preservative. |
| Volume: | 50 ml/bottle |
| Storage: | Refrigerate at 2-8°C |
| Stability: | 12 months or as indicated on label |
| Preparation: | Dilute 1:10 in distilled or deionized water before use. If the whole plate is to be used dilute 50 ml of the wash buffer concentrate in 450 ml of water. |

**2. AA E-0055** SUBSTRATE **TMB Substrate** - Ready To Use.

| | |
|---|---|
| Contents: | One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer. |
| Volume: | 16 ml/bottle |
| Storage: | Refrigerate at 2-8°C |
| Stability: | Unopened at 2-8°C until expiration date on label. |

**3. AA E-0080** STOP-SOLN **Stopping Solution** - Ready To Use.

| | |
|---|---|
| Contents: | One vial containing 1M sulfuric acid. |
| Volume: | 6 ml/bottle |
| Storage: | Refrigerate at 2-8°C |
| Stability: | Unopened at 2-8°C until expiration date on label. |
| Hazards identification: |  |

H290 May be corrosive to metals.
H314 Causes severe skin burns and eye damage.

**4. Standards** and **Controls**- Ready To Use.

Listed below are approximate concentrations, please refer to vial labels for exact concentrations:

| Cat. no. | Symbol | Standard | Concentration | Volume/Vial |
|---|---|---|---|---|
| **ME E-0301** | STANDARD A | Standard A | 0 ng/ml | 0.5 ml |
| **ME E-0302** | STANDARD B | Standard B | 1 ng/ml | 0.5 ml |
| **ME E-0303** | STANDARD C | Standard C | 5 ng/ml | 0.5 ml |
| **ME E-0304** | STANDARD D | Standard D | 10 ng/ml | 0.5 ml |
| **ME E-0305** | STANDARD E | Standard E | 20 ng/ml | 0.5 ml |
| **ME E-0306** | STANDARD F | Standard F | 50 ng/ml | 0.5 ml |
| **ME E-0307** | STANDARD G | Standard G | 100 ng/ml | 0.5 ml |
| **ME E-0351** | CONTROL 1 | Control 1 | Refer to vial labels for expected value and acceptable range! | 0.5 ml |
| **ME E-0352** | CONTROL 2 | Control 2 | | 0.5 ml |

Contents: Leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin.

Storage: Refrigerate at 2-8°C

Stability: Unopened at 2-8°C until expiration date on label.

**5. ME E-0313** ASSAY-BUFF **Assay Buffer** - Ready To Use.

Contents: One vial containing a protein-based buffer with a non-mercury preservative.

Volume: 20 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: Unopened at 2-8°C until expiration date on label.

**6. ME E-0331** 96 **Anti-Leptin Monoclonal Antibody-Coated Break Apart Well Microplate**- - Ready To Use.

Contents: One 96 well (12x8) monoclonal antibody-coated microplate in a resealable pouch with desiccant.

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

**7. ME E-0341** BIOTIN-AB **Monoclonal Anti-Leptin-Biotin Conjugate**

Contents: One bottle containing a monoclonal anti-leptin antibody conjugated to biotin in a protein-based buffer with a non-mercury preservative.

Volume: 10 ml/bottle

Storage: Refrigerate at 2-8°C

Stability: 12 months or as indicated on label.

**8. ME E-0340** CONJUGATE-CONC 50x **Streptavidin-HRP Conjugate Concentrate** – Requires Preparation **X50**

Contents: One vial containing streptavidin conjugated to horseradish peroxidase in a protein-based buffer with a non-mercury preservative.

Volume: 0.4 ml/vial

Storage: Refrigerate at 2-8°C

Stability: Unopened at 2-8°C until expiration date on label.

Preparation: Dilute 1:50 in assay buffer before use (eg. 40 µl of concentrate in 2 ml of assay buffer). If the whole plate is to be used dilute 240 µl of concentrate in 12 ml of assay buffer. Discard any that is left over.

***ASSAY PROCEDURE***

All reagents must reach room temperature before use. Standards, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

| | |
|---|---|
| **1.** | Prepare **working solutions** of the **streptavidin-HRP - conjugate** and **wash buffer.** |
| **2.** | Pipette **20 µl** of each **standard, control** and **serum samples** into correspondingly labelled wells in duplicate. |
| **3.** | Pipette **80 µl** of the **monoclonal anti-leptin-biotin conjugate** into each well. |
| **4.** | Incubate on a plate shaker (approximately 200 rpm) for **1 hour** at **room temperature**. |
| **5.** | Wash the wells **3 times** with prepared wash buffer (**300 µl**/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. |
| **6.** | Pipette **100 µl** of prepared **streptavidin-HRP** - **conjugate** into each well. |
| **7.** | Incubate on a plate shaker (approximately 200 rpm) for **30 minutes** at **room temperature**. |
| **8.** | Wash the wells **3 times** with prepared wash buffer (**300 µl**/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. |
| **9.** | Pipette **100 µl** of **TMB substrate** into each well at timed intervals. |
| **10.** | Incubate on a plate shaker for **10-15** minutes at **room temperature**. |
| **11.** | Pipette **50 µl** of **stopping solution** into each well at the same timed intervals as in step 9. |
| **12.** | Read the plate on a microwell plate reader at **450 nm** within 20 minutes after addition of the stopping solution. |

***CALCULATIONS***

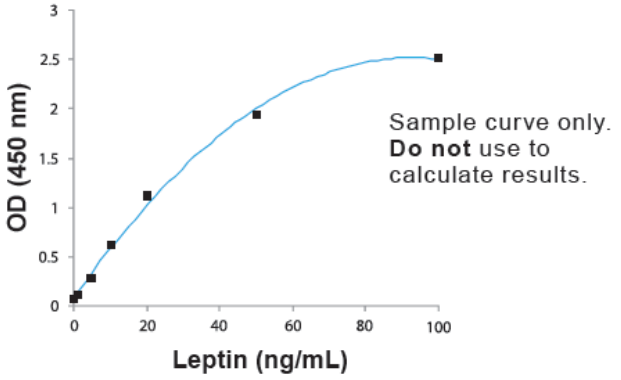
1. Calculate the mean optical density of each standard duplicate.
2. Draw a standard curve on semi-log paper with the mean optical densities on the Y-axis and the standard concentrations on the X-axis. If immunoassay software is being used, a 4-parameter or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown duplicate.
4. Read the values of the unknowns directly off the standard curve.
5. If a sample reads more than 100 ng/ml then dilute it with assay buffer at a dilution of no more than 1:8. The result obtained should be multiplied by the dilution factor.

***TYPICAL TABULATED DATA:***

Sample data only. **Do not** use to calculate results.

| Standard | OD 1 | OD 2 | Mean OD | Value (ng/ml) |
|---|---|---|---|---|
| A | 0.073 | 0.070 | 0.072 | 0 |
| B | 0.102 | 0.100 | 0.101 | 1 |
| C | 0.290 | 0.293 | 0.292 | 5 |
| D | 0.620 | 0.630 | 0.625 | 10 |
| E | 1.140 | 1.086 | 1.113 | 20 |
| F | 1.947 | 1.919 | 1.933 | 50 |
| G | 2.518 | 2.514 | 2.516 | 100 |
| Unknown | 0.275 | 0.273 | 0.274 | 4.22 |

### *TYPICAL STANDARD CURVE*




### *PERFORMANCE CHARACTERISTICS*

#### *SENSITIVITY*

The limit of detection (LoD) for Leptin is 0.50 ng/ml, as determined by use of a NCCLS protocol and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 82 blank determinations; LoB=0.42 ng/ml.

#### *SPECIFICITY*

The following substances were tested at 1000 ng/ml and exhibited no cross-reactivity:
Mouse Leptin, TNF-α, IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-9, IL-10, IL-12, IL-16, GM-CSF, CSF and EGF.

#### *INTRA-ASSAY PRECISION*

Four serum samples were assayed twenty times each on the same standard curve. The results (in ng/ml) are tabulated below:

| Sample | Mean | SD | CV% |
|---|---|---|---|
| 1 | 2.45 | 0.09 | 3.7 |
| 2 | 7.94 | 0.34 | 4.3 |
| 3 | 11.67 | 0.64 | 5.5 |
| 4 | 27.51 | 1.37 | 5.0 |

#### *INTER-ASSAY PRECISION*

Four samples were assayed ten times over a period of ten days. The results (in ng/ml) are tabulated below:

| Sample | Mean | SD | CV% |
|---|---|---|---|
| 1 | 2.71 | 0.16 | 5.9 |
| 2 | 8.24 | 0.48 | 5.8 |
| 3 | 12.01 | 0.82 | 6.8 |
| 4 | 24.98 | 1.45 | 5.8 |

#### *RECOVERY*

Spiked samples were prepared by adding defined amounts of leptin to three serum samples. The results (in ng/ml) are tabulated below:

| Sample | Observed | Expected | % Recovery |
|---|---|---|---|
| 1 Unspiked | 3.89 | - | - |
| + 3.06 | 6.28 | 6.95 | 90.4 |
| + 8.06 | 10.98 | 11.95 | 91.9 |
| + 23.00 | 25.43 | 26.95 | 94.4 |
| 2 Unspiked | 7.89 | - | - |
| + 1.06 | 8.82 | 8.95 | 98.5 |
| + 6.06 | 15.03 | 13.95 | 107.7 |
| + 21.06 | 30.32 | 28.95 | 104.7 |
| 3 Unspiked | 11.61 | - | - |
| + 4.2 | 15.71 | 15.81 | 99.4 |
| + 12.8 | 25.42 | 24.41 | 104.1 |
| + 29.5 | 41.18 | 41.07 | 100.3 |

***LINEARITY***
Three serum samples were serially diluted with leptin assay buffer. The results (in ng/ml) are tabulated below:

| Sample | Observed | Expected | % Recovery |
|---|---|---|---|
| 1 | 3.03 | - | - |
| 1:2 | 1.42 | 1.52 | 93.4 |
| 1:4 | 0.71 | 0.76 | 93.4 |
| 1:8 | 0.35 | 0.38 | 92.1 |
| 2 | 11.27 | - | - |
| 1:2 | 5.93 | 5.64 | 105.1 |
| 1:4 | 3.05 | 2.82 | 108.2 |
| 1:8 | 1.35 | 1.41 | 95.7 |
| 3 | 27.91 | - | - |
| 1:2 | 14.91 | 13.96 | 106.8 |
| 1:4 | 6.74 | 6.98 | 96.6 |
| 1:8 | 3.29 | 3.49 | 94.3 |

***COMPARATIVE STUDY***
The Leptin ELISA was compared against a leading competitor's Leptin EIA kit (Kit X).
Thirty-eight serum samples ranging from 1.05 - 75.62 ng/ml were assayed with both kits, yielding the following results:

Regression: Kit X=0.9644 (Leptin ELISA) + 1.5489
r=0.98, Kit X Mean: 21.13, LDN Mean: 20.30

***EXPECTED NORMAL VALUES***
Each laboratory should collect data and establish their own range of expected normal values.

| Group | Mean (ng/ml) | Range (ng/ml) |
|---|---|---|
| Lean Women | 7.4 | 3.7-11.1 |
| Lean Men | 3.8 | 2.0-5.6 |

Leptin values are appromiately 2.5 times higher in women than men per unit BMI.

***REFERENCES***


1. Dagogo-Jack S, et al. Plasma Leptin and Inulin Relationships in Obese and Nonobese Humans. Diabetes. 1996; 45(5):695–8.
2. Chessler SD, et al. Increased Plasma Leptin Levels Are Assosciated with Fat Accumulation in Japanese Americans. Diabetes. 1998; 47(2):239–43.
3. Heymsfi eld SB, et al. Recombinant Leptin for Weight Loss in Obese and Lean Adults: A Randomized, Controlled, Dose- Escalation Trial. JAMA. 1999; 282(16):1568–75.
4. Wiesner G, et al. Leptin is Released from the Human Brain: Infl uence of Adiposity and Gender. J Clin Endocrinol Metab. 1999; 84(7):2270–4.
5. Quinton ND, et al. Leptin Binding Activity Changes With Age: The Link Between Leptin and Puberty. J Clin Endocrinol Metab. 1999; 84(7):2336–41.
6. Considine R, et al. Serum Immunoreactive Leptin Concentration in Normal-Weight and Obese Humans. N Engl J Med. 1996; 334:292–95.
7. Maffei M, et al. Leptin Levels in Human and Rodent: Measurement of Plasma Level Leptin and obRNA in Obese and Weight Reduced Subjects. Nat Med. 1995; 1(11):1155–61.
8. Tamura T, et al. Serum Leptin Concentrations During Pregnancy and Their Relationship to Fetal Growth. Obstet Gynecol. 1998; 91(3):389–95.
9. Matsuda J, et al. Serum Leptin Concentration in Cord Blood:Relationship to Birth Weight and Gender. J Clin Endocrinol Metab. 1997; 82(5):1642–4.
10. Check JH, et al. Falsely Elevated Steroidal Assay Levels Related to Heterophile Antibodies Against Various Animal Species. Gynecol Obstet Invest. 1995; 40(2): 139–40.


**Symbols:**

| | | | | | |
|---|---|---|---|---|---|
|  | Storage temperature |  | Manufacturer |  | Contains sufficient for <n> tests |
|  | Expiry date | LOT | Batch code | | |
|  | Consult instructions for use | CONT | Content | | |
|  | Caution | REF | Catalogue number | RUO | For research use only! |