Quantitative measurement of Leptin in Human serum or plasma

1. INTRODUCTION
The DIAGNOSTIC AUTOMATION INC., Leptin (Sandwich) Enzyme Immunoassay Kit provides materials for the quantitative determination of Leptin in serum and plasma. This assay is intended for in vitro diagnostic use only.

2. PRINCIPLE OF THE TEST
The DIAGNOSTIC AUTOMATION INC., Leptin (Sandwich) ELISA is a solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The microtiter wells are coated with a monoclonal anti Leptin antibody. An aliquot of patient sample containing endogenous Leptin is incubated in the coated well with a specific rabbit anti Leptin antibody. A sandwich complex is formed. After incubation the unbound material is washed off and an anti rabbit peroxidase conjugate is added for detection of the bound Leptin. Having added the substrate solution, the intensity of color developed is proportional to the concentration of Leptin in the patient sample.

3. PRECAUTIONS
- This kit is for in vitro diagnostic use only.
- For information on hazardous substances included in the kit please refer to Material Safety Data Sheets.
- 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.
- Avoid contact with Stop Solution containing 0.5 M H₂SO₄. It may cause skin irritation and burns.
- Never pipet by mouth and avoid contact of reagents and specimens with skin and mucous membranes.
- Do not smoke, eat, drink or apply cosmetics in areas where specimens or kit reagents are handled.
- Wear disposable latex gloves when handling specimens and reagents. Microbial contamination of reagents or specimens may give false results.
- Handling should be in accordance with the procedures defined by an appropriate national biohazard safety guideline or regulation.

MICROWELL ELISA
Leptin ELISA
Cat # 1742-6
• Do not use reagents beyond expiry date as shown on the kit labels.
• All indicated volumes have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes.
• Do not mix or use components from kits with different lot numbers. It is advised not to exchange wells of different plates even if 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.
• Chemicals and prepared or used reagents have to be treated as hazardous waste according the national biohazard safety guideline or regulation.
• Safety Data Sheets for this product are available upon request directly from DIAGNOSTIC AUTOMATION INC., Instruments.

4. KIT COMPONENTS
4.1. Contents of the Kit
1. **Microtiterwells**, 12x8 (break apart) strips, 96 wells
   Wells coated with monoclonal mouse anti Leptin Antibody
2. **Standard (Standard 0-5)**, 6 vials, 200 µl, ready to use
   0, 2, 5, 25, 50 and 100 ng/ml
3. **Controls**, 2 vials, 200 µl, ready to use
   2 levels (low and high), please refer to the label for the concentrations
4. **Assay Buffer**, 1 vial, 11 ml, ready to use
5. **Antiserum**, 1 vial, 11 ml, ready to use
   Polyclonal Leptin antiserum
6. **Enzyme Complex**, 1 vial, 11ml, ready to use
   Anti Rabbit Complex containing Horseradish Peroxidase
7. **Substrate Solution**, 1 vial, 11ml, ready to use
   TMB
8. **Stop Solution**, 1 vial, 6 ml, ready to use
   contains 0.5M H₂SO₄
   Avoid contact with the stop solution. It may cause skin irritations and burns.
9. **Wash Solution**, 1 vial, 30 ml (40X concentrated)
   see „Preparation of Reagents“

Note: Additional Zero Standard (Standard 0) for Sample dilution available on request.

4.1.1 Equipment and material required but not provided
1. A microtiterplate calibrated reader (450±10 nm)(e.g. the DIAGNOSTIC AUTOMATION INC ’s Instruments Microtiterplate Reader).
2. Calibrated variable precision micropipettes.
3. Absorbent paper.
4. Aqua dest.

4.2 Storage and stability of the Kit
• When stored at 2° to 8°C unopened reagents will retain reactivity until expiration date. Do not use reagents beyond this date.
• Enzyme-Conjugate, Substrate Solution, Standards and Zero Standard must be stored at 2° to 8°C.
• Microtiter wells must be stored at 2° to 8°C. Once the foilbag has been opened, care should be taken to close it tightly again.
4.3 Preparation of Reagents
Allow all reagents and required number of strips to reach room temperature prior to use.

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.

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

4.5 Damaged Test Kits
In case of any severe damage of the test kit or components, DIAGNOSTIC AUTOMATION INC., have to be informed written, 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.

5. SPECIMEN
5.1 Specimen collection
Collect blood by venipuncture, allow to clot, and separate by centrifugation at room temperature. Do not use haemolytic, icteric or lipaemic serum.

5.2 Specimen storage
Specimens should be capped and may be stored for up to 24 hours at 2-8°C prior to assaying. Specimen 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.

5.3 Specimen dilution
If in an initial assay, a serum specimen is found to contain more than the highest standard, the specimens can be diluted 10-fold or 100 fold with Zero Standard and reassayed as described in Assay Procedure.
Example:
   a) dilution 1:10:  10 µl Serum + 90 µl Zero Standard (mix thoroughly)
   b) dilution 1:100: 10 µl dilution a) 1:10 + 90 µl Zero Standard (mix thoroughly).

6. TEST PROCEDURE
6.1 General Remarks
- All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
- Once the test has been started, all steps should be completed without interruption.
- Use new disposal plastic pipet tips for each standard, control of sample in order to avoid crosscontamination
- Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents be ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
- The present kit is adjusted to give an absorption for the highest standard > 1.200 within 10 minutes at room temperature. As a general rule the enzymatic reaction is linearly proportional to time and temperature. Therefore, if the Optical Density is too high or too low, the substrate incubation time can be decreased or increased, respectively.

6.2 Procedural Notes
- All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
• The concentration of the samples can be read directly from this standard curve. Samples with a concentration higher than that of the highest standard have to be diluted 1:10 with Zero Standard. For the calculation of the concentrations this dilution factor has to be taken into account.

6.3 Assay Procedure
1. Secure the desired number of Microtiterwells in the holder.
2. Dispense 15 µl Standards, Controls and samples with new disposable tips into appropriate wells.
3. Dispense 100 µl Assay Buffer into each well.
4. Thoroughly mix for 10 seconds. It is important to have a complete mixing in this step.
5. Incubate for 2 hours at room temperature without covering the plate.
6. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual water droplets.
   **Important note:**
   The sensitivity and precision of this assay is markedly influenced by the correct performance of the washing procedure!
7. Add 100 µl of Antiserum to each well.
8. Incubate for 30 minutes at room temperature.
9. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual water droplets.
10. Dispense 100 µl of Enzyme Complex into each well.
11. Incubate for 30 minutes at room temperature.
12. Briskly shake out the contents of the wells.
   Rinse the wells 3 times with diluted Wash Solution (300 µl per well). Strike the wells sharply on absorbent paper to remove residual water droplets.
13. Add 100 µl of Substrate Solution to each well.
14. Incubate for 15 minutes at room temperature.
15. Stop the enzymatic reaction by adding 50 µl of Stop Solution to each well.
16. Read the OD at 450±10 nm with a microtiterplate reader within 10 minutes after adding the Stop Solution.

6.4 Calculation of Results
1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Construct a standard curve by plotting the mean absorbance 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 absorbance value for each sample determine the corresponding concentration from the standard curve. Depending on experience and/or the availability of computer capability, other methods of data deduction may be employed.
4. Automated method: Computer programs using cubic spline, 4 PL (4 Parameter Logistics) or Logit-Log can generally give a good fit.
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 diluted with Zero Standard. For the calculation of the concentrations this dilution factor has to be taken into account.

Below is listed a typical example of a standard curve with the Leptin ELISA.

<table>
<thead>
<tr>
<th>Standard</th>
<th>Optical Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 0 (0 ng/ml)</td>
<td>0.08</td>
</tr>
<tr>
<td>Standard 1 (2 ng/ml)</td>
<td>0.25</td>
</tr>
<tr>
<td>Standard 2 (5 ng/ml)</td>
<td>0.50</td>
</tr>
<tr>
<td>Standard 3 (25 ng/ml)</td>
<td>1.31</td>
</tr>
</tbody>
</table>
7. ASSAY CHARACTERISTICS

7.1 Expected values
It is strongly recommended that each laboratory should determine its own normal and abnormal values.

<table>
<thead>
<tr>
<th>Standard 4 (50 ng/ml)</th>
<th>1.67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard 5 (100 ng/ml)</td>
<td>1.93</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Men</td>
</tr>
</tbody>
</table>

7.2 Specificity
The following proteins were tested for cross-reactivity of the assay:

<table>
<thead>
<tr>
<th>Component</th>
<th>Crossreactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Leptin</td>
<td>100 %</td>
</tr>
<tr>
<td>Rat Leptin</td>
<td>&lt;0.2 %</td>
</tr>
<tr>
<td>Mouse Leptin</td>
<td>&lt;0.2 %</td>
</tr>
<tr>
<td>Human Insulin</td>
<td>N.D.</td>
</tr>
<tr>
<td>Human Proinsulin</td>
<td>N.D.</td>
</tr>
<tr>
<td>Rat Insulin</td>
<td>N.D.</td>
</tr>
<tr>
<td>Human C-Peptide</td>
<td>N.D.</td>
</tr>
<tr>
<td>Glucagon</td>
<td>N.D.</td>
</tr>
<tr>
<td>IGF-I</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

N.D.: Not detectable

7.3 Sensitivity
The minimum detectable concentration of Leptin by this assay is estimated to be 1.0 ng/ml

7.4 Accuracy

Quality Control
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 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 DIAGNOSTIC AUTOMATION INC., directly.
7.5 Precision
Data can be obtained on request.

8. LIMITATIONS OF USE
8.1 Interfering Substances
Any improper handling of samples or modification of this test might influence the results. Interferences caused by improper sample handling are explained in the chapters ‘Specimen - Collection’.

8.2 High-Dose-Hook Effect
Data can be obtained on request.

9. LEGAL ASPECTS
9.1 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 DIAGNOSTIC AUTOMATION INC.

9.2 Therapeutical Consequences
Therapeutical consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under point 9.1. 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 therapeutical consequences be derived.

The test result itself should never be the sole determinant for deriving any therapeutical consequences.

9.3 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 point 9.2. 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.

10. REFERENCES

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Revision Date: 5-13-2005