Leptin ELISA

For the quantitative determination of leptin in human serum

For Research Use Only. Not For Use In Diagnostic Procedures.

Catalog Number: 11-LEPHU-E01
Size: 96 wells
Version: 8.0 October 26, 2010 - ALPCO August 20, 2012
INTENDED USE
This kit is for the quantitative determination of leptin in human serum by an enzyme immunoassay method. This kit is for research use only.

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 microwell plate 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 colored product that is directly proportional to the amount of leptin present. The enzymatic reaction is terminated by the addition of the stop solution, converting the blue color to a yellow color. 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.

RESEARCH APPLICATIONS
Human leptin is a 16 kDa, 146 amino acid residue, non-glycosylated polypeptide. Leptin is encoded by the OB gene. Its major source is adipose tissue, and its circulating concentrations indirectly reflect body fat stores. Plasma or serum concentrations of leptin are increased in obese humans and strongly correlate with the degree of adiposity as expressed by percentage of body fat or body mass index. The recently discovered hormone leptin contributes to the regulation of energy balance by informing the brain of the amount of adipose tissue in the body. The brain may then make the appropriate adjustments in either energy intake or expenditure. Many areas of leptin physiology remain to be investigated. The roles of leptin in metabolism, insulin sensitivity, as a potential therapeutic modality for weight loss as well as involvement in endocrine function are active areas of research. While the future for leptin as a therapeutic agent is not clear, its involvement in many areas of physiology undoubtedly makes this a new hormone which requires extensive study in the future to understand its physiology.

PROCEDURAL CAUTIONS AND WARNINGS
1. This kit is intended for research use only. It is not for use in diagnostic procedures.
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 samples or kit reagents are being handled.
   - Wear protective clothing and disposable gloves when handling the samples and kit reagents.
   - Wash hands thoroughly after performing the test.
   - Avoid contact with eyes; use safety glasses. If contact occurs, 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 calibrator 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 confidence limits.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. All kit reagents and samples should be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of reagents and samples.
10. Improper procedural techniques, imprecise pipetting, incomplete washing, or improper reagent storage may be indicated when assay values for the controls do not reflect established ranges.
11. When reading the microplate, the presence of bubbles in the microwells 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 colorless if properly stored. Instability or contamination may be indicated by the development of a blue color, in which case it should not be used.
13. When dispensing the substrate and stop 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 samples 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.
5. This kit is for research use only. It is not for use in diagnostic procedures.

SAFETY: CAUTIONS AND WARNINGS

POTENTIALLY BIOHAZARDOUS MATERIAL
All serum samples should be considered potential biohazards and handled with the appropriate precautions.

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

SERUM COLLECTION AND STORAGE
Approximately 0.1 mL of serum is required per duplicate determination. Collect 4-5 mL of blood into an appropriately labeled 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 samples as potentially biohazardous materials and take appropriate precautions when handling.

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

**REAGENTS PROVIDED**
1. Anti-Leptin Monoclonal Antibody Coated Microwell Plate-Break Apart Wells
   Contents: One 96 well (12 x 8) monoclonal antibody-coated microwell plate in a resealable pouch with desiccant.
   Storage: Refrigerate at 2-8°C
   Stability: Unopened at 2-8°C until expiration date on label.

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

3. Streptavidin-HRP Conjugate Concentrate
   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 (e.g., 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 remaining solution.

4. Leptin Calibrators
   Contents: Six vials containing leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin. Listed below are approximate concentrations, please refer to bottle labels for exact concentrations.

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>Concentration</th>
<th>Volume/Vial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calibrator A</td>
<td>0 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator B</td>
<td>1 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator C</td>
<td>5 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator D</td>
<td>10 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator E</td>
<td>20 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator F</td>
<td>50 ng/mL</td>
<td>0.5 mL</td>
</tr>
<tr>
<td>Calibrator G</td>
<td>100 ng/mL</td>
<td>0.5 mL</td>
</tr>
</tbody>
</table>

Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.
5. Controls
Contents: Two vials containing leptin in a protein-based buffer with a non-mercury preservative. Prepared by spiking buffer with a defined quantity of leptin. Refer to bottle label for expected value and acceptable range.
Volume: 0.5 mL/vial
Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.

6. Wash Buffer Concentrate
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: Unopened at 2-8°C until expiration date 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.

7. Assay Buffer
Contents: One bottle 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.

8. TMB Substrate
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.

9. Stop Solution
Contents: One bottle containing 1 M sulfuric acid.
Volume: 6 mL/bottle
Storage: Refrigerate at 2-8°C
Stability: Unopened at 2-8°C until expiration date on label.

ASSAY PROCEDURE
All reagents must reach room temperature before use. Calibrators, controls, and 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 calibrator, control, and serum sample into the correspondingly labeled 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 diluted wash buffer (300 μL/well for each wash) and tap the plate firmly against absorbent paper to ensure that it is dry. (The use of an automatic washer is highly recommended.)
6. Pipette 100 µL of diluted 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 again in the same manner as in step 5.
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 stop 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 the addition of the stop solution.

CALCULATIONS
1. Calculate the mean optical density of each calibrator duplicate.
2. Draw a calibrator curve on semi-log paper with the mean optical densities on the Y-axis and the calibrator concentrations on the X-axis. If immunoassay software is being used, a 4- or 5-parameter curve is recommended.
3. Calculate the mean optical density of each unknown sample duplicate.
4. Read the values of the unknowns directly off the calibrator 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

<table>
<thead>
<tr>
<th>Calibrator</th>
<th>OD 1</th>
<th>OD 2</th>
<th>Mean OD</th>
<th>Value (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.073</td>
<td>0.070</td>
<td>0.072</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>0.102</td>
<td>0.100</td>
<td>0.101</td>
<td>1</td>
</tr>
<tr>
<td>C</td>
<td>0.290</td>
<td>0.293</td>
<td>0.292</td>
<td>5</td>
</tr>
<tr>
<td>D</td>
<td>0.620</td>
<td>0.630</td>
<td>0.625</td>
<td>10</td>
</tr>
<tr>
<td>E</td>
<td>1.140</td>
<td>1.086</td>
<td>1.113</td>
<td>20</td>
</tr>
<tr>
<td>F</td>
<td>1.947</td>
<td>1.919</td>
<td>1.933</td>
<td>50</td>
</tr>
<tr>
<td>G</td>
<td>2.518</td>
<td>2.514</td>
<td>2.516</td>
<td>100</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.275</td>
<td>0.273</td>
<td>0.274</td>
<td>4.22</td>
</tr>
</tbody>
</table>

TYPICAL CALIBRATOR CURVE
Sample curve only. Do not use to calculate results.
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 (\(\alpha\)) less than 5% and false negatives (\(\beta\)) 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-\(\alpha\), 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 calibrator curve. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.45</td>
<td>0.09</td>
<td>3.7</td>
</tr>
<tr>
<td>2</td>
<td>7.94</td>
<td>0.34</td>
<td>4.3</td>
</tr>
<tr>
<td>3</td>
<td>11.67</td>
<td>0.64</td>
<td>5.5</td>
</tr>
<tr>
<td>4</td>
<td>27.51</td>
<td>1.37</td>
<td>5.0</td>
</tr>
</tbody>
</table>

INTER-ASSAY PRECISION
Four samples were assayed ten times over a period of ten days. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2.71</td>
<td>0.16</td>
<td>5.9</td>
</tr>
<tr>
<td>2</td>
<td>8.24</td>
<td>0.48</td>
<td>5.8</td>
</tr>
<tr>
<td>3</td>
<td>12.01</td>
<td>0.82</td>
<td>6.8</td>
</tr>
<tr>
<td>4</td>
<td>24.98</td>
<td>1.45</td>
<td>5.8</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed</th>
<th>Expected</th>
<th>%Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Unspiked</td>
<td>3.89</td>
<td>6.95</td>
<td>90.4</td>
</tr>
<tr>
<td></td>
<td>6.28</td>
<td>11.95</td>
<td>91.9</td>
</tr>
<tr>
<td></td>
<td>10.98</td>
<td>26.95</td>
<td>94.4</td>
</tr>
<tr>
<td>2 Unspiked</td>
<td>7.89</td>
<td>8.95</td>
<td>98.5</td>
</tr>
<tr>
<td></td>
<td>8.82</td>
<td>13.95</td>
<td>107.7</td>
</tr>
<tr>
<td></td>
<td>15.03</td>
<td>28.95</td>
<td>104.7</td>
</tr>
<tr>
<td>3 Unspiked</td>
<td>11.61</td>
<td>15.81</td>
<td>99.4</td>
</tr>
<tr>
<td></td>
<td>15.71</td>
<td>24.41</td>
<td>104.1</td>
</tr>
<tr>
<td></td>
<td>25.42</td>
<td>41.07</td>
<td>100.3</td>
</tr>
</tbody>
</table>
LINEARITY
Three serum samples were serially diluted with leptin assay buffer. The results (in ng/mL) are tabulated below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Observed</th>
<th>Expected</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:2</td>
<td>3.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:4</td>
<td>1.42</td>
<td>1.52</td>
<td>93.4</td>
</tr>
<tr>
<td>1:8</td>
<td>0.71</td>
<td>0.76</td>
<td>94.7</td>
</tr>
<tr>
<td>1:2</td>
<td>11.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:4</td>
<td>5.93</td>
<td>5.64</td>
<td>105.1</td>
</tr>
<tr>
<td>1:8</td>
<td>3.05</td>
<td>2.82</td>
<td>108.2</td>
</tr>
<tr>
<td>1:2</td>
<td>27.91</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:4</td>
<td>14.91</td>
<td>13.96</td>
<td>106.8</td>
</tr>
<tr>
<td>1:8</td>
<td>6.74</td>
<td>6.98</td>
<td>96.6</td>
</tr>
<tr>
<td></td>
<td>3.29</td>
<td>3.49</td>
<td>94.3</td>
</tr>
</tbody>
</table>

COMPARATIVE STUDY
This 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 (11-LEPHU-E01) + 1.5489
r=0.98
Kit X Mean: 21.13
11-LEPHU-E01 Mean: 20.30

EXPECTED NORMAL VALUES
As for all assays, each laboratory should collect data and establish its own range of expected normal values.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean (ng/mL)</th>
<th>Range (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lean Women</td>
<td>7.4</td>
<td>3.7-11.1</td>
</tr>
<tr>
<td>Lean Men</td>
<td>3.8</td>
<td>2.0-5.6</td>
</tr>
</tbody>
</table>

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

REFERENCES