Leptin (human), ELISA kit

Catalog #: ADI-900-028A

96 Well Enzyme-Linked Immunosorbent Assay Kit
For use with serum, plasma and culture supernates
USE FOR RESEARCH PURPOSES ONLY

Unless otherwise specified expressly on the packaging, all products sold hereunder are intended for and may be used for research purposes only and may not be used for food, drug, cosmetic or household use or for the diagnosis or treatment of human beings. Purchase does not include any right or license to use, develop or otherwise exploit these products commercially. Any commercial use, development or exploitation of these products or development using these products without the express written authorization of Enzo Life Sciences, Inc. is strictly prohibited. Buyer assumes all risk and liability for the use and/or results obtained by the use of the products covered by this invoice whether used singularly or in combination with other products.

LIMITED WARRANTY; DISCLAIMER OF WARRANTIES

These products are offered under a limited warranty. The products are guaranteed to meet all appropriate specifications described in the package insert at the time of shipment. Enzo Life Sciences’ sole obligation is to replace the product to the extent of the purchasing price. All claims must be made to Enzo Life Sciences, Inc., within five (5) days of receipt of order. THIS WARRANTY IS EXPRESSLY IN LIEU OF ANY OTHER WARRANTIES OR LIABILITIES, EXPRESS OR IMPLIED, INCLUDING WARRANTIES OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, AND NON-INFRINGEMENT OF THE PATENT OR OTHER INTELLECTUAL PROPERTY RIGHTS OF OTHERS, AND ALL SUCH WARRANTIES (AND ANY OTHER WARRANTIES IMPLIED BY LAW) ARE EXPRESSLY DISCLAIMED.

TRADEMARKS AND PATENTS

Several Enzo Life Sciences products and product applications are covered by US and foreign patents and patents pending. Enzo is a trademark of Enzo Life Sciences, Inc.

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.
# TABLE OF CONTENTS

- **Introduction** ................................................................. 2
- **Principle** ................................................................. 3
- **Materials Supplied** ..................................................... 4
- **Storage** ................................................................. 5
- **Other Materials Needed** ........................................ 5
- **Reagent Preparation** ............................................... 6
- **Sample Handling** ...................................................... 7
- **Assay Procedure** ..................................................... 12
- **Calculation of Results** ............................................. 13
- **Typical Results** ..................................................... 14
- **Typical Standard Curve** ........................................... 14
- **Performance Characteristics** .................................... 15
- **References** ............................................................ 16
- **Contact Information** ............................................... 18

---

Please read entire booklet before proceeding with the assay.

Carefully note the handling and storage conditions of each kit component.

Please contact Enzo Life Sciences Technical Support if necessary.
INTRODUCTION

The Leptin (human), ELISA kit is a complete kit for the quantitative determination of leptin in serum, plasma, and culture supernates of human origin. Please read the entire kit insert before performing this assay.

The adipokine leptin is a hormone secreted predominantly by adipose tissue that signals through leptin receptors in the hypothalamus to decrease appetite and increase energy expenditure. Binding of leptin to the long-form of the leptin receptor in the hypothalamus reduces neuropeptide Y (NPY) and agouti-related protein (AgRP) activity, while stimulating anorexigenic pro-opiomelanocortin (POMC) neuron activity to reduce appetite. In peripheral tissues, leptin antagonizes insulin signaling, increases fatty acid oxidation, decreases insulin production in pancreatic β cells, and promotes fertility. Its expression in adipocytes can be regulated in a paracrine fashion by other adipokines such as IL-6 (stimulation) and TNF-α (inhibition).

Leptin deficient mice display severe insulin resistance, obesity, and decreased fertility, all of which are reversible by administration of exogenous leptin. In contrast, elevated circulating levels of leptin are associated with increased obesity, indicative of an acclimated state of leptin resistance which is not well understood. Evidence also links leptin to cardiovascular disease, as it mediates numerous pro-inflammatory and pro-atherogenic effects such as increased endothelin-1 (ET-1) expression, accumulation of reactive oxygen species (ROS), secretion of monocyte chemoattractant protein-1 (MCP-1), and increased sympathetic vascular tone and blood pressure.
PRINCIPLE

- Samples and standards are added to wells coated with a monoclonal antibody specific for human leptin. The plate is then incubated.

- The plate is washed, leaving only bound human leptin on the plate. A yellow solution of biotinylated polyclonal antibody specific for human leptin is then added. This binds the human leptin captured on the plate. The plate is then incubated.

- The plate is washed to remove excess antibody. A blue solution of streptavidin conjugated to horseradish peroxidase (HRP) is added to each well, binding to the biotinylated antibody. The plate is again incubated.

- The plate is washed to remove excess HRP conjugate. TMB Substrate solution is added. An HRP-catalyzed reaction generates a blue color in the solution.

- Stop solution is added to stop the substrate reaction. The resulting yellow color is read at 450nm. The amount of signal is directly proportional to the level of human leptin in the sample.
MATERIALS SUPPLIED

1. **Assay Buffer 17**
   100ml, Catalog No. 80-1573
   Tris buffered saline containing BSA and detergent

2. **Recombinant Leptin (human) Standard**
   Catalog No. 80-1807
   Two vials containing 2000 pg of recombinant human leptin

3. **Leptin (human) Clear Microtiter Plate**
   One plate of 96 wells, Catalog No. 80-1806
   A clear plate of break-apart strips coated with a mouse monoclonal antibody specific for human leptin

4. **Leptin (human) ELISA Antibody**
   10ml, Catalog No. 80-1809
   A yellow solution of biotinylated monoclonal antibody specific for human leptin

5. **Leptin (human) ELISA Conjugate**
   10ml, Catalog No. 80-1810
   A blue solution of streptavidin conjugated to horseradish peroxidase

6. **Wash Buffer Concentrate**
   100ml, Catalog No. 80-1287
   Tris buffered saline containing detergents

7. **TMB Substrate**
   10ml, Catalog No. 80-0350
   A solution of 3,3'5,5' tetramethylbenzidine (TMB) and hydrogen peroxide

8. **Stop Solution 2**
   10ml, Catalog No. 80-0377
   A 1N solution of hydrochloric acid in water

9. **Leptin (human) Assay Layout Sheet**
   1 each, Catalog No. 30-0254

10. **Plate Sealer**
    2 each, Catalog No. 30-0012
STORAGE
All components of this kit are stable at 4°C until the kit’s expiration date.

MATERIALS NEEDED BUT NOT SUPPLIED
1. Deionized of distilled water
2. Precision pipets for volumes between 5µl and 1,000µl
3. Repeater pipet for dispensing 100µl
4. Disposable beakers for diluting buffer concentrates
5. Graduated cylinders
6. Lint-free paper for blotting
7. Microplate reader capable of reading at 450nm
8. Software for extrapolating sample values from optical density readings utilizing a four parameter logistic curve fit
REAGENT PREPARATION

1. Wash Buffer

Prepare the wash buffer by diluting 50ml of the supplied Wash Buffer Concentrate with 950ml of deionized water. This can be stored at room temperature until the kit’s expiration, or for 3 months, whichever is earlier.

2. Preparation of Leptin Standard Curve

Reconstitute the 2000 pg lyophilized standard vial with 1ml of Assay Buffer 17 for a final concentration of 2000pg/ml. This is tube #1. Vortex to mix. Let the reconstituted standard sit at room temperature for 5 minutes. Vortex again. Label six tubes #2 through #7. Pipet 500µl of Assay Buffer 17 into each tube. Pipet 500µl of the 2000pg/ml standard to tube #2. Vortex to mix. Add 500µl of tube #2 to tube #3. Vortex to mix. Continue this for tubes #3 through #7.

Diluted standards should be used within 1 hour of preparation.

The concentrations of the standards are labeled above.
SAMPLE HANDLING

Human serum, Na EDTA plasma, and culture supernates are suitable for use in this assay. Other sample matrices have not been validated. Prior to running the assay, samples should be slowly brought to 4°C and centrifuged, if necessary, to remove debris. Due to differences in samples, users must determine the optimal sample dilution for their particular experiments. Below are examples of the linearity experiments performed to determine the optimal sample dilution for these samples.

Serum and Plasma:

A minimum dilution of 1:20 into the assay buffer is required for most serum samples, while a 1:10 dilution into the assay buffer is required for most plasma samples. These dilutions were based on a pool of either serum or plasma (n=6 for each matrix). Actual minimal required dilution may vary from sample to sample. A range of 1:4 to 1:64 has been observed. Starting dilutions will also vary from sample to sample due to differences in leptin concentrations. **Most serum and plasma samples will require a 1:50 dilution into Assay Buffer 17 to fall within the dynamic range of the assay.**

Culture Supernates:

No dilution is required for most culture supernates with or without 10% fetal bovine serum (FBS). Changes in binding associated with non-validated culture media may be corrected by using the same non-conditioned media as the standard diluent.
Dilutional Linearity

The minimum required dilution for several common sample matrices was determined by serially diluting samples into the assay buffer and identifying the dilution at which linearity is observed. Non-conditioned Dulbecco’s Modified Eagle’s Medium (DMEM) with or without 10% fetal bovine serum (FBS) was spiked with recombinant human leptin and diluted in the assay buffer. Human serum and plasma were pooled, then diluted in the assay buffer to produce values within the dynamic range of the assay.

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Culture Media (DMEM)</th>
<th>Culture Media (DMEM + 10% FBS)</th>
<th>Serum</th>
<th>Na EDTA Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neat</td>
<td>91%</td>
<td>91%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1:2</td>
<td>90%</td>
<td>89%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1:4</td>
<td>90%</td>
<td>85%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1:5</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>116%</td>
</tr>
<tr>
<td>1:8</td>
<td>91%</td>
<td>89%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1:10</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>108%</td>
</tr>
<tr>
<td>1:16</td>
<td>90%</td>
<td>91%</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>1:20</td>
<td>---</td>
<td>---</td>
<td>104%</td>
<td>112%</td>
</tr>
<tr>
<td>1:40</td>
<td>---</td>
<td>---</td>
<td>102%</td>
<td>108%</td>
</tr>
<tr>
<td>1:80</td>
<td>---</td>
<td>---</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>1:160</td>
<td>---</td>
<td>---</td>
<td>100%</td>
<td>---</td>
</tr>
</tbody>
</table>
**Spike and Recovery**

After diluting each individual sample to read within the dynamic range of the assay, recombinant human leptin was spiked at high, medium, and low concentrations. The average recovery of the standard in each spiked samples was compared to the recovery of identical spikes in the assay buffer. The mean and range percent recovery at the three concentrations are indicated below for each matrix.

<table>
<thead>
<tr>
<th>Sample Matrix (# of samples)</th>
<th>Dilution</th>
<th>Spike Concentration (pg/ml)</th>
<th>Recovery of Spike (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEM (n = 1) Neat</td>
<td></td>
<td>1800</td>
<td>89% (n/a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>90% (n/a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>98% (n/a)</td>
</tr>
<tr>
<td>DMEM + 10% FBS (n = 1) Neat</td>
<td></td>
<td>1800</td>
<td>103% (n/a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>101% (n/a)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>113% (n/a)</td>
</tr>
<tr>
<td>Serum (n = 6) 1:5 - 1:200</td>
<td></td>
<td>1500</td>
<td>105% (102-107%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>108% (94-122%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>82% (10-156%)</td>
</tr>
<tr>
<td>Na EDTA Plasma (n = 6) 1:10 - 1:200</td>
<td></td>
<td>1500</td>
<td>92% (86-95%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>250</td>
<td>105% (92-119%)</td>
</tr>
</tbody>
</table>
Parallelism

A parallelism experiment was carried out to determine if the recombinant leptin standard accurately determines leptin concentrations in biological matrices. To assess parallelism, values for each matrix were obtained from a standard curve using four parameter logistic curve fitting. The observed concentration was plotted against the dilution factor. Parallelism of the curves demonstrates that the antibody binding characteristics are similar enough to allow the accurate determination of human leptin levels in diluted samples.
Sample Values

Levels obtained from individual serum and Na EDTA plasma samples were assessed in the kit. The results are shown below.

<table>
<thead>
<tr>
<th>Sample Type (# of samples)</th>
<th>Range (pg/ml)</th>
<th>Average (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (n=10)</td>
<td>728 - 65,304</td>
<td>29,575</td>
</tr>
<tr>
<td>Na EDTA Plasma (n=10)</td>
<td>3,187 - 94,786</td>
<td>30,464</td>
</tr>
</tbody>
</table>

Serum and Plasma Preparation

1. Collect whole blood in appropriate tubes for either serum or plasma.
2. Allow serum to clot for 30 minutes.
3. Centrifuge at 1000 x g for 15 minutes at 4°C.
4. Place supernatants in a clean tube.
5. The supernates may be aliquoted and stored at or below -20°C, or used immediately in the assay.
6. Avoid repeated freeze-thaw cycles.
ASSAY PROCEDURE

Refer to the Assay Layout Sheet to determine the number of wells to be used. Remove the wells not needed for the assay and return them, with the desiccant, to the mylar bag and seal. Store unused wells at 4°C.

1. Pipet 100µl of Assay Buffer 17 into the S0 (0pg/ml standard) wells.
2. Pipet 100µl of standards #1 through #7 to the bottoms of the appropriate wells.
3. Pipet 100µl of the samples to the bottoms of the appropriate wells.
4. Seal the plate and incubate for 1 hour without shaking at room temperature.
5. Empty the contents of the wells and wash by adding 400µl of wash buffer to every well. Repeat 3 more times for a total of 4 washes. After the final wash, empty or aspirate the wells and firmly tap the plate on a lint free paper towel to remove any remaining wash buffer.
6. Pipet 100µl of yellow antibody into each well except the blank.
7. Seal the plate and incubate for 1 hour without shaking at room temperature.
8. Wash as above (Step 5).
9. Pipet 100µl of blue conjugate to each well except the blank.
10. Seal the plate and incubate for 30 minutes without shaking at room temperature.
11. Wash as above (Step 5).
12. Pipet 100µl of substrate solution into each well.
13. Seal the plate and incubate for 30 minutes without shaking at room temperature.
14. Pipet 100µl of stop solution into each well.
15. After blanking the plate reader against the substrate only blank, read optical density at 450nm. If plate reader is not capable of adjusting for the blank, manually subtract the mean OD of the substrate blank from all readings.

Bring all reagents to room temperature for at least 30 minutes prior to opening.

All standards and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of the antibody, conjugate and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

All standards and samples should be run in duplicate.

Pre-rinse each pipet tip with reagent. Use fresh pipet tips for each sample, standard, and reagent.

Pipet the reagents to the sides of the wells to avoid possible contamination.

Prior to the addition of the antibody, conjugate and substrate, ensure there is no residual wash buffer in the wells. Remaining wash buffer may cause variation in assay results.

Bring all reagents to room temperature for at least 30 minutes prior to opening.
CALCULATION OF RESULTS

Several options are available for the calculation of the concentration of human leptin in the samples. We recommend that the data be handled by an immunoassay software package utilizing a four parameter logistic curve fitting program. If data reduction software is not readily available, the concentrations can be calculated as follows:

1. Calculate the average Net OD for each standard and sample by subtracting the average blank OD from the average OD for each standard and sample.

   \[ \text{Average Net OD} = \text{Average OD} - \text{Average Blank OD} \]

2. Plot the average Net OD for each standard versus human leptin concentration in each standard. Approximate a straight line through the points. The concentration of unknown samples can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be re-analyzed using a different dilution.
**TYPICAL RESULTS**

The results shown below are for illustration only and should not be used to calculate results from another assay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Net OD</th>
<th>Leptin (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S0</td>
<td>0.017</td>
<td>0</td>
</tr>
<tr>
<td>S1</td>
<td>2.481</td>
<td>2000</td>
</tr>
<tr>
<td>S2</td>
<td>1.357</td>
<td>1000</td>
</tr>
<tr>
<td>S3</td>
<td>0.626</td>
<td>500</td>
</tr>
<tr>
<td>S4</td>
<td>0.276</td>
<td>240</td>
</tr>
<tr>
<td>S5</td>
<td>0.132</td>
<td>125</td>
</tr>
<tr>
<td>S6</td>
<td>0.068</td>
<td>62.5</td>
</tr>
<tr>
<td>S7</td>
<td>0.041</td>
<td>31.25</td>
</tr>
</tbody>
</table>
PERFORMANCE CHARACTERISTICS

Specificity
The cross reactivities of related compounds were determined by diluting cross reactants in the assay buffer at a concentration of 200 ng/ml. The cross reactants were then measured in the assay.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross Reactivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human leptin</td>
<td>100%</td>
</tr>
<tr>
<td>Rat leptin</td>
<td>&lt;0.01%</td>
</tr>
<tr>
<td>Mouse leptin</td>
<td>&lt;0.01%</td>
</tr>
</tbody>
</table>

Sensitivity
The sensitivity, or limit of detection of the assay, is 23.4pg/ml. The sensitivity was determined by interpolation at two standard deviations above the mean signal of background (0pg/ml) using data from nine standard curves.

Precision
Intra-assay precision was determined by assaying 20 replicates of three buffer controls containing leptin in a single assay.

<table>
<thead>
<tr>
<th>pg/ml</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>634.8</td>
<td>4.4</td>
</tr>
<tr>
<td>165.9</td>
<td>7.4</td>
</tr>
<tr>
<td>78.6</td>
<td>13.4</td>
</tr>
</tbody>
</table>

Inter-assay precision was determined by measuring buffer controls of varying leptin concentrations in multiple assays over several days.

<table>
<thead>
<tr>
<th>pg/ml</th>
<th>%CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>665.0</td>
<td>3.7</td>
</tr>
<tr>
<td>181.7</td>
<td>7.1</td>
</tr>
<tr>
<td>99.7</td>
<td>15.2</td>
</tr>
</tbody>
</table>
REFERENCES