Catalog #80968

V.4

Leptin ELISA
Kit Instructions

For the quantitative determination of
leptin in human serum

Catalog #80968
96 Assays

For research use only. Not for use in diagnostic procedures.

Crystal Chem, Inc.
955 Busse Road
Elk Grove Village, IL 60007, USA
Tel: (630) 889-9003 Fax: (630) 889-9021
E-mail: sales@crystalchem.com
Order online: www.crystalchem.com

Catalog #80968

TABLE OF CONTENTS

A. Intended Use 1

B. Introduction 1

C. Principles of the Assay 1

D. Kit Storage 1

E. Assay Materials

E.1. Materials provided 1

E.2. Materials required by not provided 2

F. Assay Precautions 2

G. Maximizing Kit Performance 2

H. Sample Collection 2

I. Assay Procedure

I.1. Preparation of reagents 2

I.2. Assay procedure 3

I.3. Determining the leptin concentration 3

J. Performance characteristics

J.1. Assay range 4

J.2. Precision 4

Warranty 4

A. Intended Use

The Leptin ELISA kit is for the quantitative determination of leptin in human serum. Please read the complete kit insert before performing this assay. The kit is for RESEARCH USE ONLY. It is not intended for use in diagnostic procedures.

B. Introduction

Obesity is a significant contributing factor in various adult diseases such as diabetes, cardiac disease, etc. This fact, combined with the increasing prevalence of obesity in the human population, has resulted in increased research on the underlying impact and cause of obesity.

In 1994, leptin, obese gene product, was identified from the investigation of ob/ob mouse. Leptin is a protein of about 16 kDa, which is expressed in adipose tissue, and promotes weight loss by suppressing appetite and stimulating metabolism. As a result, the accurate measurement of leptin is becoming increasingly important as obesity research intensifies.

C. Principle of the Assay

The Leptin ELISA kit is a sandwich ELISA. An unknown amount of leptin present in the sample binds with anti-leptin antibodies bound to the microplate. After washing to remove unbound proteins, biotinylated anti-leptin antibodies are added to bind to the leptin present in the wells. After a subsequent washing step, a streptavidin-HRP conjugate reacts to form a complex on the surface. After a final washing step, a TMB substrate is added, and the concentration of leptin is determined by color intensity.

D. Kit Storage

1. Upon receipt of the leptin ELISA kit, store it at 2-8°C and avoid light exposure (do not freeze the kit or hold it at temperatures above 25°C).
2. The kit should not be used after the expiration date.

E. Assay Materials

E.1. Materials provided

TABLE 1 Contents of the kit

Mark	Description	Amount
MIC	Antibody-coated Microplate (12 x 8)	1 pack
STD1-7	Standards	7 vials
CON1-2	Controls	2 vials
AB CONJ	Antibody-Biotin Conjugate	10 mL
HRP	HRP Conjugate (50x Concentrate)	400 µL
WASH	Wash Buffer (10X Concentrate)	50 mL
BUF	Assay Buffer	20 mL
SUB	Substrate Solution	16 mL
STOP	Stop Solution	6 mL

E.2. Materials required but not provided

Micropipettes and disposable tips
Deionized or distilled water
Microplate reader (capable of reading at 450 nm)
Orbital shaker

F. Assay Precautions

1. Only appropriately-trained personnel should use the kit. Laboratory personnel should wear suitable protective clothing. All chemicals and reagents should be considered potentially hazardous. Avoid ingestion and contact with skin and eyes.
2. Some assay components may contain human sourced materials. Accordingly, all assay components should be handled as if potentially infectious using safe laboratory procedures.
3. Do not use the reagents after the expiration date.
4. Reagents are light sensitive and should be protected from sunlight.
5. Do not let the substrate or stop solution come in contact with metal parts including aluminum foil.

G. Maximizing Kit Performance

1. Given the small volumes required (20 µL), pipetting should be done as carefully as possible. A high quality 50 µL or better precision pipette should be used for such volumes. Drops of liquid adhering to the outside of the pipette tips should be removed by wiping to ensure the highest degree of accuracy.
2. In order to prevent the microplate wells from drying out and to get the best results, samples and reagents should be dispensed quickly into the wells.
3. Each standard, control, and sample should be assayed in duplicate.
4. The same sequence of pipetting and other operations should be maintained in all procedures.
5. Do not mix reagents that have different lot numbers.

H. Sample Collection

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. Serum samples can be capped and stored at 2-8°C for up to 24 hours prior to assaying. For longer term storage, samples should be stored at -20°C. Avoid repeated freeze-thaw cycles of samples. Thawed samples should be inverted several times prior to testing. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum. Samples containing azide or thimerosal are not compatible with this kit.

I. Assay Procedure

All reagents, unless otherwise noted, are stable until the expiration date at 2-8°C once opened.

I.1. Preparation of reagents

1. Antibody-coated microplate
   Provided as ready to use. Protect from moisture.
2. Standards 1-6
   Standards are provided in liquid form with concentrations ranging from 0 ng/mL to 100 ng/mL. Standards are provided in the following approximate concentrations: 0, 1, 5, 10, 20, 50, and 100 ng/mL, and *exact values are listed on each bottle.*

3. Controls 1-2
   Controls are provided in liquid form (0.5mL) with target value and ranges included on their labels.
4. Antibody-Biotin Conjugate
   Provided as ready to use.
5. HRP Conjugate (50x Concentrated)
   The HRP Conjugate has to be diluted 1:50 with Assay Buffer. For example, 40 µL of HRP Conjugate must be diluted with 1.96 mL of Assay Buffer.
6. Wash Buffer (10X Concentrated)
   The wash buffer has to be diluted 1:10 with distilled or deionized water prior to use. For example, 50 mL of wash buffer must be diluted with 450 mL of distilled or deionized water. Dilute only as needed.
7. Assay Buffer
   Provided as ready to use.
8. Substrate Solution
   Provided as ready to use.
9. Stop Solution
   Provided as ready to use.

I.2. Assay procedure

Prior to running the assay, all reagents should be brought to room temperature for at least 30 minutes. Reagents should be stored at 2-8°C immediately after use. Before use, mix the reagents thoroughly by gentle agitation or swirling.

1. In each well, add 20 µL of sample, standard, or control.
2. In each well, add 80 µL of Antibody-Biotin conjugate.
3. Incubate on a plate shaker (200 rpm) for 60 mins at ambient temperature.
4. Aspirate well contents and wash three times using 300 µL of prepared Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
5. Add 100 µL of working HRP Conjugate in each well.
6. Incubate on a plate shaker (200 rpm) for 30 mins at ambient temperature.
7. Aspirate well contents and wash three times using 300 µL of prepared Wash Buffer per well. After each wash, remove any remaining solution by inverting and tapping the plate firmly on a clean paper towel.
8. Add 100 µL of Substrate Solution in each well.
9. Incubate the plate for 15 mins at ambient temperature on a plate shaker (200 rpm) in a dark room.
10. Stop the reaction by adding 50 µL of Stop Solution per well.
11. Measure the optical density within 20 minutes using a plate reader at 450 nm.

I.3. Determining the Leptin concentration

1. Using computer software, construct the leptin calibration curve by plotting the mean optical density for each standard on the Y axis versus the corresponding leptin concentration on the X axis. A four or five parameter curve fit is suitable for the evaluation.
   Note: *A calibration curve should be plotted every time the assay is performed.*
2. Leptin concentrations in the samples or controls are interpolated using the calibration curve and mean optical density for each sample. The leptin concentration is expressed in ng/mL.
   Note: *Samples with a reading higher than 100 ng/mL should be diluted with the assay buffer and rerun. Extrapolated values need to be multiplied by the dilution factor to calculate the leptin concentration. Do not use a dilution factor higher than 1:8.*

J. Performance characteristics

J.1. Assay range

The Leptin ELISA Kit has an assay range from 1 – 100 ng/mL.

J.2. Precision

The assay has a within-run and total precision of CV $\leq$ 10%.

Warranty

Crystal Chem Inc. makes no warranty of any kind, either expressed or implied, except that the materials from which its products are made are of standard quality. Buyer assumes all risk and liability resulting from the use of this product.

THERE IS NO WARRANTY OF MERCHANTABILITY OF THE PRODUCTS, OR THAT SUCH PRODUCTS ARE FIT FOR ANY PARTICULAR PURPOSE. CRYSTAL CHEM INC'S LIABILITY SHALL NOT EXCEED THE RETURN OF THE PURCHASE PRICE, AND UNDER NO CIRCUMSTANCES SHALL CRYSTAL CHEM INC. BE LIABLE FOR SPECIAL OR CONSEQUENTIAL DAMAGES, OR EXPENSES ARISING DIRECTLY OR INDIRECTLY FROM THE USE OF THIS PRODUCT.