OriGene Technologies Inc.
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Human Leptin ELISA Kit

Catalog Number: EA100299

Assay Principle

The OriGene Human LEP Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human LEP with a 96-well strip plate that is pre-coated with antibody specific for LEP. The detection antibody is a biotinylated antibody specific for LEP. The capture antibody is monoclonal antibody from mouse and the detection antibody is polyclonal antibody from goat. The kit contains recombinant Human LEP with immunogen: Expression system for standard: E.coli; Immunogen sequence: V22-C167. The kit is analytically validated with ready-to-use reagents.

To measure Human LEP, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450nm is linearly proportional to Human LEP in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human LEP in the sample.

Overview

Product Name	Human Leptin ELISA Kit
Reactive Species	Human
Size	96wells/kit, with removable strips.
Description	Human Leptin ELISA Kit PicoKine™ (96 Tests). Quantitate Human LEP in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA). Sensitivity: 10pg/ml.
Sensitivity	<10 pg/ml *The sensitivity or the minimum detectable dose (MDD) is the lower limit of target protein that can be detected by the kit. It is determined by adding two standard deviations to the mean O.D. value of twenty (20) blank wells and calculating the corresponding concentration.
Detection Range	62.5 pg/ml – 4000 pg/ml
Storage Instructions	Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Uniprot ID	A4D0Y8

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Technical Details

Capture/Detection Antibodies	The capture antibody is monoclonal antibody from mouse, the detection antibody is polyclonal antibody from goat.
Specificity	Natural and recombinant Human LEP
Immunogen	Expression system for standard: E.coli; Immunogen Sequence: V22 – C167
Cross Reactivity	This kit is for the detection of Human LEP. No significant cross-reactivity or interference between LEP and its analogs was observed. This claim is limited by exiting techniques therefore cross-reactivity may exist with untested analogs.

Notice Before Application

Please read the following instructions before starting the experiment.

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see sections Sample Preparation) and that you have sufficient sample volume for the assay.
5. When first using the kit, appropriate validation steps should be taken before using valuable samples. Confirm kit adequately detects the antigen in your intended sample types by running control samples.
6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see section Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the Kit, spin tubes and bring down all components to the bottom of tubes.
9. Don't let 96-well plate dry, for dry plate will inactivate active components on plate.
10. Don't reuse tips and tubes to avoid cross contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature and kit age can cause variation in binding. Variations in sample collection, processing and storage may cause sample value differences.

Kit Components/Materials Provided

Description	Quantity	Volume	Storage of opened/reconstituted material
Anti-Human LEP Pre-coated 96-well strip microplate	**1**	**12 strips of 8 wells**	**Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Human LEP Standard	**2**	**10 ng/tube**	**Discard the LEP stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.**
Human LEP Biotinylated antibody (100x)	**1**	**100 µl**	**May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.**
Avidin-Biotin-Peroxidase Complex (100x)	**1**	**100 µl**	
Sample Diluent	**1**	**30ml**	

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Antibody Diluent	***1***	***12ml***	
Avidin-Biotin-Peroxidase Diluent	***1***	***12ml***	
Color Developing Reagent (TMB)	***1***	***10ml***	
Stop Solution	***1***	***10ml***	
Wash Buffer (25 x)	***1***	***20 ml***	
Plate Sealers	***4***	***Piece***	

Required Materials That Are Not Supplied

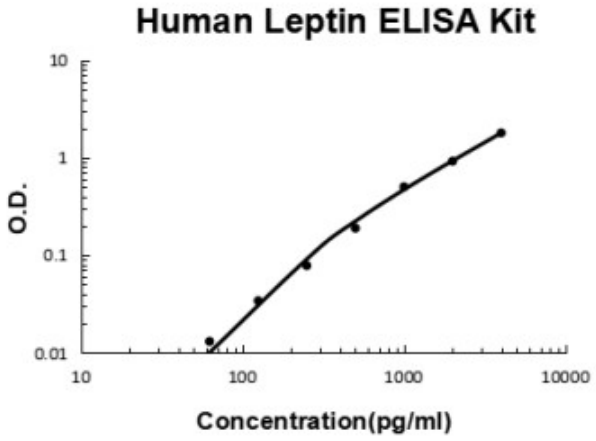
Microplate Reader capable of reading absorbance at 450nm.
Automated plate washer (optional)
Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions. Multichannel pipettes are recommended for large amount of samples.
Deionized or distilled water.
500ml graduated cylinders.
Test tubes for dilution.

Human LEP ELISA Kit (EA100299) Standard Curve Example

Highest O.D. value might be higher or lower than in the example. The experiment result is statistically significant if the highest O.D. value is no less than 1.0.

Concentration (pg/ml)	***0***	***62.5***	***125***	***250***	***500***	***1000***	***2000***	***4000***
O.D.	***0.002***	***0.015***	***0.036***	***0.080***	***0.191***	***0.505***	***0.923***	***1.801***

Human LEP ELISA Kit standard curve



A standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

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Intra/Inter Assay Variability

OriGene spend great efforts in documenting lot to lot variability and make sure our assay kits produce robust data that are reproducible.

Intra-Assay Precision (Precision within an assay): *Three samples of known concentration were tested on one plate to assess intra-assay precision.*
Inter-Assay Precision (Precision across assays): *Three samples of known concentration were tested in separate assays to assess inter-assay precision*

	Intra-Assay Precision			*Inter-Assay Precision*		
Sample	1	2	3	1	2	3
n	16	16	16	24	24	24
Mean(pg/ml)	81	485	1057	*75*	444	996
Standard deviation	6.15	32.49	54.96	8	35.52	69.72
CV(%)	7.6%	6.7%	5.2%	8.4%	8%	7%

Reproducibility

To assay reproducibility, three samples with differing target protein concentrations were assayed using four different lots.

Lots	*Lot1 (pg/ml)*	*Lot2 (pg/ml)*	*Lot3 (pg/ml)*	*Lot4 (pg/ml)*	*Mean (pg/ml)*	*Standard Deviation*	*CV (%)*
Sample 1	81	83	*84*	*87*	83	2.16	2.6%
Sample 2	485	*5396*	*463*	476	490	*28.93*	5.9%
Sample 3	1057	1101	*1114*	*1116*	*1097*	23.8	2.1%

**number of samples for each test n=16.*

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Preparation Before The Experiment

Item	Preparation
All reagents	Bring all reagents to room temperature (18-25°C) prior to use. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also the TMB incubation time estimate (15-25min) is based on 37°C. Please do not equilibrate ununsed plate well strips to room temperature. They should be sealed and stored in the original packaging.
Wash buffer	Prepare 500 ml of working Wash Buffer by diluting the suspended 20 ml Wash Buffer (25 x) with 480 ml of deonized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
Biotinylated Anti-Human LEP antibody	It is recommended to prepare this reagent immediately prior to use by diluting the Human LEP Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Avidin-Biotin-Peroxidase Complex	It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin-Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mix gently and thoroughly and use within 2 hours of generation.
Human LEP Standard	It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human LEP standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions.
Microplate	The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.
Samples	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.

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Dilution of Human LEP Standard

1. Number tubes 1-8. Final Concentrations to be Tube # 1 –4000pg/ml, #2 –2000pg/ml, #3 –1000pg/ml, #4 – 500pg/ml, #5 – 250pg/ml, #6 – 125pg/ml, #7 – 62.50pg/ml, #8 – 0.0 (Blank).
2. To generate standard #1, add 400µl of the reconstituted standard stock solution of 10ng/ml and 600µl of sample diluent to tube #1 for a final volume of 1000µl. Mix thoroughly.
3. Add 300 µl of sample diluent to tubes # 2-7.
4. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
5. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
6. Continue the serial dilution for tube #4-7.
7. Tube #8 is a blank standard to be used with every experiment.

Sample Preparation and Storage

These sample collection instructions and storage conditions are intended as a general guideline and the sample stability has not been evaluated.

Sample dilution ratios should be determined by a pilot study (run a serial dilution of samples and see which dilution ratio results in the idea O.D., near the middle of the standard range). In general, high concentration samples can be dilutioned by 1:100, mid concentration samples 1:10, low concentration samples 1:2 or neat.

Sample Type	Procedure
Cell culture supernatants	Clear sample of particulates by centrifugation, assay immediately or store samples at -20°C.
Serum	Use a serum separator tube (SST) and allow serum to clot at room temperature for about four hours. Then, centrifuge for 15 min at approximately 1,000 x g. assay immediately or store samples at -20°C.
Plasma	Collect plasma using heparin, EDTA or Citrate as an anticoagulant. Centrifuge for 15 min at approximately 1,000 x g. Assay immediately or store samples at -20°C. *Note: it is important to not use anticoagulants other than the ones described above to treat plasma for other anticoagulants could block the antibody binding site.
Cell lysate	Lyse the cells , make sure there are no visible cell sediments. Centrifuge cell lysates at approx. 10000 x g for 5 min. Collect the supernatant. Assay immediately or store samples at -20°C.

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Sample Collection Notes

1. Is is recommend that samples are immediately used upon preparation
2. Avoid repeated freeze/thaw cycles for all samples.
3. In the event that a sample type not listed above is intended to be used with the kit, it is recommended that the customer conduct validation experiments in order to be confident in the results.
4. Due to chemical interference, the use of tissue or cell extraction samples prepared by chemical lysis buffers may result in inaccurate results.
5. Due to factors including cell viability, cell number or sampling time, samples from cell culture supernatant may to be detected by the kit.
6. Samples should be brought to room temperature (18-25 °C) before performing the assay without the use of extra heating.
7. Sample concentrations should be predicted before being used in the assay. If the samples concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

Sample Dilution

The target protein concentration should be estimated and appropriate sample dilutions should be selected such that the final protein concentration lies near the middle of the linear dynamic range of the assay.

Dilute the sample using provided diluent buffer. Pilot tests using a dilution series of each sample type may be necessary. The sample must be mixed thoroughly with sample diluent.

Assay protocol

It is recommended that all reagents and materials be equilibrated to room temperature (18-25 °C) prior to the experiment (see Preparation Before The Experiment if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the sample diluent buffer into the control well (Zero well). At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at RT (or 90 min. at 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human LEP antibody to each well.
7. Cover with plate sealer and incubate for 90 minutes at RT (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer.
   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
   c. Repeat steps a-b 2 additional times.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer.
    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
    b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).
    c. Repeat steps a-b 4 additional times.
11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard

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wells, while the remaining standards remain clear.)

12. *Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.*
13. *Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450nm.*

Assay Protocol Notes

1. *Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.*
2. *Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.*
3. *Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.*
4. *Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.*
5. *Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.*
6. *Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.*
7. *Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking OD readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.*
8. *Reaction Time Control: Control reaction time should be strictly followed as outlined.*
9. *Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.*
10. *To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.*

Data Analysis

Average the duplicate readings for each standard, sample, and control. Subtract the average zero standard O.D. reading.

It is recommended that a standard curve be created using computer software to generate a four parameter logistic (4-PL) curve-fit. A free program capable of generating a four parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay

Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative OD against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data.

For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Background on LEP

Leptin (or obese, OB) is a circulating hormone that is expressed abundantly and specifically in the adipose tissue. Human ob protein is a 166-amino acid polypeptide with a putative signal sequence and is 84 and 83% homologous to the mouse and rat ob proteins, respectively. Northern blot analysis reveals that OB RNA is present at a high level in adipose tissue but at much lower levels in placenta and heart. Indeed, Leptin is first described as an adipocyte-derived signalling factor, which, after interaction with its receptors, induces a complex response including control of bodyweight and energy expenditure. Leptin seems in addition to its role in metabolic control to have important roles in reproduction and neuroendocrine signalling. Leptin is a powerful inhibitor of bone formation in vivo. Comparative mapping of mouse and human DNA indicates that the ob gene is located within a region of mouse chromosome 6 that is homologous to a portion of human chromosome 7q. The standard product used in this kit is recombinant human Leptin.