Human Leptin ELISA Kit

Cat#: orb735166 (ELISA Manual)

Introduction

Leptin is a polypeptide hormone encoded by obesity genes. It is an encoded protein that uses a mutation found in genetic maps to cause obesity. Leptin master To be expressed in adipose tissue, the hypothalamus plays an important role in regulating energy consumption and intake in the body. Discovery and cloning of ob gene A major breakthrough in the study of re-regulation mechanism. Human leptin has a molecular weight of 16 kDa, contains 146 amino acid residues, and is highly hydrophilic. Mature human leptin and mouse and rat leptin protein There are 87% and 84% homology. It has been found that human leptin plays a role in mouse and rat systems. The expression of leptin is very limited, and it is mainly produced by white adipose tissue. The secretion of leptin from white adipose tissue is directly proportional to the body fat content. The amount of body fat is the main factor affecting the secretion of leptin. In addition, changes in energy intake, sleep, body temperature, gender, own circadian rhythm, other Hormones can affect its secretion and expression. Generally speaking, the normal blood leptin level has been found to be lower than the nanogram/ml concentration. The Leptin receptor OB-R is a bridge for Leptin to act on the hypothalamus. Leptin inhibits food intake and increases energy consumption through specific receptors in the hypothalamus. Consumption plays an important role in fat accumulation and weight regulation. The level of Leptin in plasma is related to the amount of adipose tissue. When it drops, the level of Leptin in the plasma also drops. Leptin mainly regulates body fat deposition, regulates body fat metabolism, and reduces weight through three ways: suppress appetite, reduce energy intake Into; increase energy consumption; inhibit fat synthesis. Among them, the first two pathways play a central role through the binding of leptin to its hypothalamic receptors. Achieved. As LPR is found to be widely distributed in various peripheral tissues, the latter approach may be related to its peripheral effects. As a hormone, leptin has a wide range of biological effects: inhibit food intake, promote energy consumption, regulate metabolism, and affect hormone secretion and production. Reproduction, immunity and vascular proliferation, etc., and these effects require binding to specific receptors. Changing circulating leptin levels can be used to study atherosclerosis Sclerosis, growth hormone deficiency, and eating disorders such as anorexia nervosa. The results of a clinical trial of subcutaneous injection of leptin for weight loss conducted by Heymsfield et al. showed that leptin is effective for weight loss, but it is not ideal. The effect of the low-dose group Not obvious, only the high-dose group (0.30 mg/kg, 24 weeks) weight loss was statistically significant. For this reason, the focus of the treatment of obesity with leptin should be on How to improve the sensitivity of leptin and overcome leptin resistance. Some scholars have found that if leptin is mutated, it loses its biological activity, but the knot is retained. The ability to combine receptors can be used in animal husbandry to increase animal fat.

Detection principle

This experiment uses a double antibody sandwich ELISA. Pre-coated the ELISA plate with anti-human Leptin monoclonal antibody, and add appropriately diluted samples and standards. The Leptin will bind to its monoclonal antibody to wash away free components; add biotinylated anti-human Leptin antibody, and the

anti-human Leptin antibody will bind to the monoclonal antibody Human Leptin binds to form an immune complex, washing away free components; adding horseradish peroxidase-labeled avidin, biotin and avidin Heterosexual binding, wash away the unbound enzyme conjugate; add color developer, if there is Leptin in the reaction well, horseradish peroxidase will make the colorless color reagent appear Blue; yellow with stop solution. Measure the OD value at 450nm, the concentration of Leptin is proportional to the OD450 value, which can be calculated by drawing a standard curve Calculate the concentration of Leptin in the specimen.

Kit components

Kit components	96 wells	48 holes	Preparation
1a Standard	2 sticks	1 stick	Dilute according to the instructions
1b Standards and specimen diluents	4 bottles	2 bottles	Ready to use
2a Concentrated biotinylated antibody	2 sticks	1 stick	Dilute according to the label
2b Biotinylated Antibody Diluent	1 bottle	1 bottle	Ready to use
3a Concentrated enzyme conjugate (protected from light)	2 sticks	1 stick	Dilute according to the label
3b Enzyme Conjugate Diluent	1 bottle	1 bottle	Ready to use
4 Concentrated washing liquid 20×	1 bottle	1 bottle	Dilute according to the label
Chromogenic agent (avoid light)	1 bottle	1 bottle	Ready to use
Stop solution	1 bottle	1 bottle	Ready to use
Antibody coated slats	8×12	8×6	Ready to use
Sealing tape	4 sheets	2 sheets	Ready to use
Manual	1 serving	1 serving	

Storage conditions

Other experimental materials (not provided, but can be purchased with assistance):
1. Microplate reader (450nm)
2. High-precision adjustable pipettes and tips: 0.5-10, 2-20, 20-200, 200-1000μl; when there are many samples to be tested at a time, it is best to use a multi-channel pipette.
3. Automatic plate washer or bottle washer
4. 37℃ thermostats
5. Double distilled or deionized water
6. Graph paper
7. Measuring cylinder

Precautions

1. The kit is stored at 2-8°C. Except for the reconstituted standard, other components cannot be frozen.
2. The amount of concentrated biotinylated antibody (2a) and concentrated enzyme conjugate (3a) is very small, and the bumps and possible inversion during transportation may cause the liquid to stain the tube wall or bottle cap. Make Please centrifuge before use to make the liquid attached to the tube wall or bottle cap settle to the bottom of the tube.
3. To avoid cross-contamination, please use disposable tips.
4. The stop solution and the developer are corrosive. Avoid direct contact with the skin and mucous membranes. Once contact with these liquids, please rinse with plenty of water as soon as possible.
5. Use a clean plastic container to prepare the washing solution, and thoroughly mix the various components and samples in the kit before use.
6. When washing the ELISA plate, pat it dry, do not put the absorbent paper directly into the ELISA reaction well to absorb water.
7. Do not use reagents from other sources to mix or substitute the components of this product. Kit components of different batch numbers cannot be mixed. Please use this product within the expiry date.
product.
8. In the test, it is recommended to make double or triple wells when testing standards and samples. The order of adding reagents should be the same to ensure that all reaction wells are incubated at the same time.
The same.
9. Thorough mixing is particularly important for the results of the reaction; it is best to use a micro-oscillator (use the lowest frequency for oscillation).
10. Avoid drying of the microtiter plate during the operation. Drying will quickly inactivate the biological components on the microtiter plate and affect the results of the experiment.
11. Properly dilute the sample so that the sample value falls within the range of the standard curve. According to the high, medium and low content of the factor to be tested, it is recommended to use 1:100, 1:10,
Dilute the sample 1:2. If the OD value of the sample is higher than the highest standard, increase the dilution appropriately and repeat the test.
12. Differences in standard dilutions, operators, pipetting methods, washing methods, incubation time and temperature, and kit batches may cause poor results
different.
13. This method can effectively eliminate the interference of soluble receptors, binding proteins and other factors in biological samples.

Sample collection, processing and preservation methods

1. Serum: Use a pyrogen- and endotoxin-free test tube. After collecting the blood, coagulate at room temperature for 30 minutes, centrifuge at 1000×g for 10 minutes, and separate the serum carefully.

2. Plasma: Collect plasma with EDTA, citrate, and heparin as anticoagulants, and centrifuge at 1000×g for 15 minutes within 30 minutes to remove particles.

3. Cell supernatant: Centrifuge at 1000×g for 10 minutes to remove particles and polymers.

4. Storage: If the sample is not tested immediately, please aliquot it in a single dose and store it at -20℃-70℃ to avoid repeated freezing and thawing. Try to avoid using hemolysis or High blood lipid samples. If the serum contains a lot of particles, centrifuge or filter to remove them before testing; thawing at room temperature, do not store at 37°C or higher Heat to thaw.

5. Dilution: According to the actual situation, the specimen can be diluted by an appropriate multiple (it is recommended to do a preliminary experiment to determine the dilution multiple).

Note: It is recommended that normal human serum or plasma samples be diluted **1:2**.

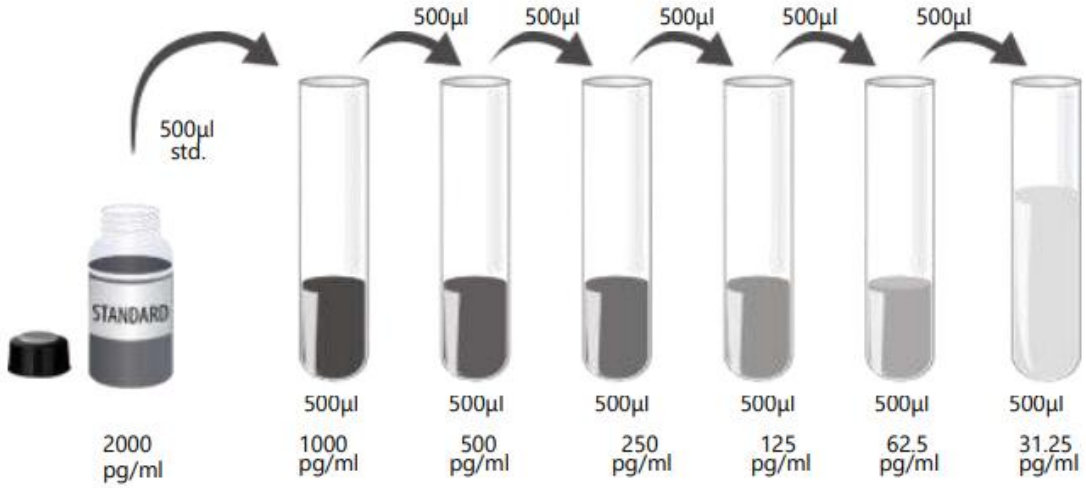
Reagent preparation

1. Take out the kit from the refrigerator 30 minutes in advance and equilibrate to room temperature.

2. Washing buffer: The concentrated washing liquid taken out of the refrigerator may have crystals. This is a normal phenomenon. After heating and shaking gently to completely dissolve the crystals Reconstituted. The concentrated washing solution was diluted with double distilled water (1:20). Return the unused ones to 4°C.

3. Standard product: Add 1.0ml of standard product/specimen diluent (1b) to the freeze-dried standard product (1a). After it is completely dissolved, let it stand for 15 minutes to mix (concentration is 2000pg/ml), and then dilute as needed, as shown in the figure below (it is recommended to use the following concentrations for the standard curve: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/ml). The diluted standard products shall not be reused. The unused standard products should be divided into one-time use and placed in-Store at 20～-70℃, one-time use, avoid repeated freezing and thawing.

Standard dilution method:



4. Biotinylated antibody working solution: calculate the total dosage according to 100µl per well, and prepare an extra 100-200µl. Diluent with biotinylated antibody
(2b) Dilute and concentrate the biotinylated antibody (2a) (1:100). It's best to use it now. (Refer to the table below for the dilution method)

Number of slats used	Concentrated enzyme conjugate		Enzyme Conjugate Diluent
12	110μL	+	10890μL
10	90μL	+	8910μL
8	70μL	+	6930μL
6	50μL	+	4950μL
4	33μL	+	3267μL
2	17μL	+	1683μL
1	9μL	+	891μL

Steps

1. Prepare various solutions according to the above preparations.
2. Determine the number of plates required according to the number of samples to be tested and the number of standards, and add 1 hole as a blank control hole. Separate the specimens and different concentrations Add standards (100μl/well) to the corresponding wells (only add standard/sample diluent to zero wells), seal the reaction wells with sealing tape, and incubate in a 37°C incubator 90 minutes (except blank control wells).
3. Wash the plate 4 times: (1) Automatic plate washer: 350μl of washing liquid is required to be injected, and the interval between injection and suction is 15-30 seconds. (2) Manually wash the plate: Dump the inside of the hole Liquid, add 350μl of washing solution to each well, let it stand for 30 seconds, shake off the liquid, and pat dry on a thick stack of absorbent paper.
4. Add biotinylated antibody working solution (100μl/well). Seal the reaction wells with sealing tape and incubate at 37°C for 60 minutes **(**except the blank control wells).
5. Wash the plate 4 times.
6. Add working solution of enzyme conjugate (100μl/well). Seal the reaction wells with sealing tape and incubate at 37°C for 30 minutes (except the blank control wells).
7. Wash the plate 4 times.
8. Add 100μl/well of chromogenic reagent, protect from light, and incubate at 37℃ for 10-20 minutes.
9. Add 100μl/well of stop solution, and measure the OD450 value immediately after mixing (within 5 minutes).

Operation flow chart

Prepare reagents and samples
Add standard and specimen 100μl/well, 37℃, 90min
Wash the plate 4 times
Add biotinylated antibody working solution 100μl/well, 37℃, 60min
Wash the plate 4 times
Add enzyme conjugate working solution 100μl/well, 37℃, 30min
Wash the plate 4 times
Add 100μl/well of developer, avoid light at 37℃, 10-20min
Add 100μl/well of stop solution, after mixing, measure OD450 value immediately

Tips for operation

1. When preparing various reagents, mix them thoroughly, but avoid a large amount of foam, so as to avoid adding a large amount of bubbles when adding samples and causing sample addition errors.
2. To avoid cross-contamination, remember to replace the tip in time when adding different concentrations of standards, different samples, and different reagents.
3. In order to ensure accurate results, the reaction wells must be sealed with new sealing tape before each incubation.
4. Before adding the color developing agent, it should remain colorless. Do not use the color developing solution that has turned blue. The optimal color development time is very important to the standard curve. It can be seen that the first 3-4 holes have a gradient of blue color, and the difference between the latter 3-4 holes is not obvious, and the zero hole can be terminated without blue appearance.
5. Make a standard curve for each test, and appropriately dilute or concentrate the sample according to the content of the factor to be tested in the sample. It is best to do a preliminary experiment.

Result judgment

1. The OD value of each standard and specimen should be subtracted from the OD value of the blank well. If a duplicate hole is made, find the average value.
2. Use the computer software to use the absorbance OD value as the ordinate (Y) and the corresponding Leptin standard concentration as the abscissa (X) to generate the corresponding standard curve.
The Leptin content of the sample can be converted to the corresponding concentration from the standard curve according to its OD value.
3. If the OD value of the sample is higher than the upper limit of the standard curve, it should be appropriately diluted and re-tested. When calculating the concentration, the sample content should be multiplied by the dilution factor.
4. Reference data:

Standard concentration **(pg/ml)**	**OD** value **1**	**OD** value **2**	average value	Correction value
0	0.058	0.056	0.057	——
31.25	0.065	0.067	0.066	0.009
62.5	0.083	0.089	0.086	0.020
125	0.135	0.135	0.135	0.078
250	0.278	0.280	0.279	0.222
500	0.650	0.664	0.657	0.600
1000	1.371	1.360	1.365	1.308
2000	2.251	2.232	2.241	2.184

The data is for reference only, the best color rendering time will be different for different users

Repeatability of results

The coefficient of variation between the plates and the plates was less than 10%.

Sensitivity

The lowest human Leptin dose is less than 15pg/ml. The method for determining the lowest detectable quantity: add two standard deviations to the average OD value of 20 zero standards, and then Calculate the corresponding concentration.

Specificity

This kit can detect both natural and recombinant human Leptin. Specific tests are performed in parallel at 50ng/ml, and none of them react with the following cytokines and proteins.

Recombinant Human Cytokine	Recombinant Mouse Cytokine	Other proteins
G-CSF	IL-1α	bovine FGF acidic
GM-CSF	IL-1β	bovine FGF basic
IL-1α	IL-3	human PDGF
IL-1β	IL-4	porcine PDGF
IL-2	IL-5	human TGF-β1
IL-3	IL-6	porcine TGF-β1
IL-4	IL-7	porcine TGF-β2
IL-5	IL-10	
IL-6	IL-13	
IL-8	Leptin	
IL-9	LIF	
IL-10	MIP-1α	
IL-12	MIP-1β	
TGF-β1	TNF-α	
TGF-β3		
TNF-α		
TNF-β		
IFN-γ		

References

1 Halaas, JL et al. (1995) Science 269:543.
2 Zhang, Y. et al. (1994) Nature 372:425.
3. Cohen, SL et al. (1996) Nature 382:589.
4. Ogawa, Y. et al. (1995) J. Clin. Invest. 96:1647.
5. Verploegen, SABW et al. (1997) FEBS Lett. 405:237.
6. Satoh, N. et al. (1997) Neurosci. Lett. 224:149.
7. Leroy, P. et al. (1996) J. Biol. Chem. 271:2365.
8 Schwartz, MW et al. (1996) Nature Med. 2:589.
9. Shimizu, H. et al. (1997) Int. J. Obesity 21:536.
10. Segal, KR et al. (1996) Diabetes 45:988.
11. Lonnqvist, F. and M. Schalling (1997) Curr. Opin. Endocrinol. Diab. 4:164.
12. Rainwater, DL et al. (1997) Atherosclerosis 132:237.
13. Fisker, S. et al. (1997) Metabolism 46:812.
14. Licinio, J. (1997) Mol. Psychiatry 2:267.
15. Hebebrand, J. et al. (1997) Mol. Psychiatry 2:330.
16. Heymsfield, B. et al. (1999) Am Med Assoc1568:1575.