# Mouse Leptin ELISA Kit

# Cat#: orb735292 (Use Manual)

Introduction

Leptin (LP) is a protein hormone secreted by adipose tissue. Leptin is the product encoded by the mouse obesity gene. Mutations in this gene block the synthesis of leptin, which in turn leads to obesity and diabetes, as well as decreased activity, metabolism and body temperature. LP is composed of 166 or 167 amino acid residues, and its amino terminus has a 21 amino acid signal peptide, so the LP secreted to the outside of the cell is a 145 or 146 peptide with a molecular weight of 16 KD. The structure of LP is highly conserved. Compared with rat LP, mouse LP has only 6 amino acid residues different, and its homology is as high as 96%, the homology with human LP is 84%, and the homology between human and rat LP is also as high as 83%. . This structural homology is the biological basis for the cross-species effect of LP. LP has no post-translational modifications such as glycosylation and sulfhydrylation, but can form intramolecular disulfide bonds after cleaving the signal peptide, making the entire molecule spherical.
Like other hormones, LP needs to bind to specific receptors to exert its biological effects. The mouse LP receptor (ob-R) is localized in db containing The gene has a 5.1cmol gap on chromosome 4. It exists in the choroid plexus and other tissues including the hypothalamus, such as the brain, liver, kidney, heart, lung, adipose tissue and the surface of islet cells. LP is expressed in subcutaneous fat, omental fat, retroperitoneal fat and mesenteric adipose tissue of human body, and subcutaneous fat is most expressed, and its main function is to regulate the deposition of body fat. It is mainly achieved by suppressing appetite, reducing energy intake, increasing energy consumption, and inhibiting fat synthesis.

Detection principle

Double antibody sandwich ELISA was used in this experiment. The microtiter plate is pre-coated with anti-mouse Leptin monoclonal antibody, and moderately diluted samples and standards are added, in which Leptin will bind to its monoclonal antibody to wash away free components; add biotinylated anti-mouse Leptin antibody, anti- The mouse Leptin antibody binds to the mouse Leptin bound to the monoclonal antibody to form an immune complex, and the free components are washed away; the horseradish peroxidase-labeled avidin is added, and the biotin is specifically bound to the avidin. Wash off the unbound enzyme conjugates; add chromogenic reagent, if there is Leptin in the reaction well, horseradish peroxidase will make the colorless chromogenic reagent appear blue; add stop solution to turn yellow. The OD value was measured at 450nm, and the Leptin concentration was proportional to the OD450 value. The Leptin concentration in the sample could be calculated by drawing a standard curve.

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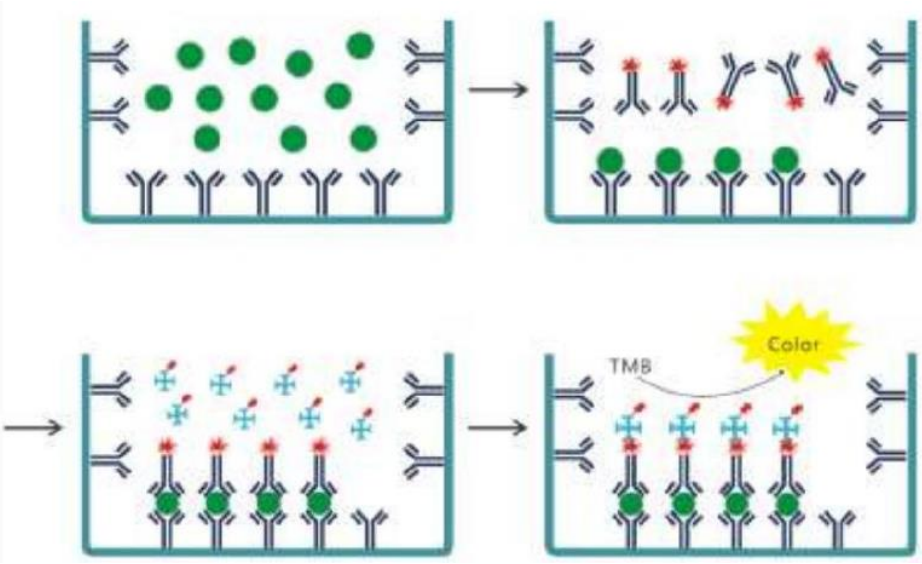



Schematic diagram of detection principle

## Kit Components

| Kit Components | 96 holes | 48 holes | formulate |
|---|---|---|---|
| 1a Standard | 2 sticks | 1 stick | Dilute according to 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 on the bottle |
| 2b Biotinylated Antibody Diluent | 1 bottle | 1 bottle | ready to use |
| 3a Concentrate enzyme conjugate (protect from light) | 2 sticks | 1 stick | Dilute according to the label on the bottle |
| 3b Enzyme Conjugate Diluent | 1 bottle | 1 bottle | ready to use |
| 4 Concentrated washing solution 20× | 1 bottle | 1 bottle | Dilute according to the label on the bottle |
| Color developer (protect from 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 | |

If you find any components in the above table are damaged or missing after receiving the kit, please contact our customer service at 400-7060-959 or tech@4abio.com in time. We will solve related problems for you in time.

Storage conditions

| | | |
|---|---|---|
| Unopened kit | | Store at 4°C and use within the expiration date. |
| already start seal up or Heavy new dissolve untie of try agent | 1b Standards and Specimen Diluents | The whole box can be stored at 4°C for 1 month.<br>2a Concentrated Biotinylated Antibody and 3a Concentrated Enzyme Conjugates require ready-to-use match. |
| | 2a Concentrated biotinylated antibody (100×) | |
| | 2b Biotinylated Antibody Diluent 3a Concentrated | |
| | Enzyme Conjugate (100× protected from light) | |
| | 3b Enzyme Conjugate Diluent | |
| | 4 Concentrated washing solution 20× | |
| | Color developer (protect from light) | |
| | Stop solution | Store at 4°C or room temperature |
| | Standard | After redissolving, repackage and store at -20°C for one month to avoid repeated freezing and thawing. dilution<br>The latter standard should be discarded after use and should not be reused. |
| | Antibody Coated Slats | The slats not used in the experiment should be immediately put back in the packaging bag, sealed and dry<br>Store at 4°C. |

The above storage conditions are required to be within the shelf life of the kit.
Other experimental materials (not provided, but available for purchase) :
1. Microplate reader (450nm)
2. High precision adjustable pipettes and tips: 0.5-10, 2-20, 20-200, 200-1000ÿl; when testing a large number of samples at one time, it is best to use multi-channel pipetting device. 3. Automatic plate washer or bottle washing 4. 37°C incubator 5. Double distilled water or deionized water 6. Graph paper 7. Graduated cylinder

Precautions

1. The kit is stored at 2-8°C. Except for the reconstituted standard, other components cannot be frozen. 2. The volume of concentrated biotinylated antibody (2a) and concentrated enzyme conjugate (3a) is very small, and bumps and possible inversions during transportation will cause the liquid to stick to the tube wall or bottle cap. Make Centrifuge before use to allow the liquid adhering to the tube wall or cap to settle to the bottom of the tube.
3. To avoid cross-contamination, use disposable tips. 4. The stop solution and developer are corrosive. Avoid direct contact with skin and mucous membranes. Once in 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, it should be fully patted dry, and do not put the absorbent paper directly into the ELISA reaction well to absorb water. 7. Do not mix or replace the components of this product with reagents from other sources. Kit components of different batches cannot be mixed.

Please use this product within the expiration date. product.
8. It is recommended to use double or triple wells for the detection of standards and samples in the test. The order of adding reagents should be the same to ensure that all reaction wells are incubated at the same time. same room.
9. Thorough mixing is especially important for the results of the reaction, preferably using a micro-shaker (using the lowest frequency for shaking). 10. Avoid
drying the ELISA plate during the operation. Drying will quickly inactivate the biological components on the ELISA plate and affect the experimental results. 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 measured, 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 result in 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

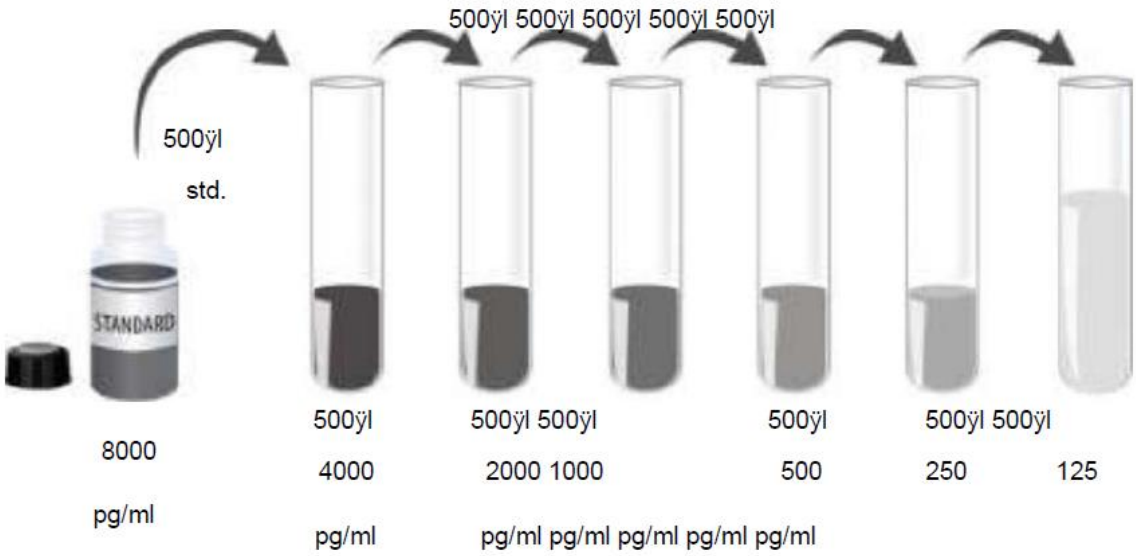
1. Serum: Use a test tube without pyrogen and endotoxin. After collecting blood, coagulate at room temperature for 30 minutes, centrifuge at 1000 × g for 10 minutes, and carefully separate the serum. 2. Plasma: EDTA, citrate, and heparin were used as anticoagulants to collect plasma, and the particles were removed by centrifugation at 1000 × g for 15 minutes within 30 minutes of collection.
3. Cell supernatant: centrifuge at 1000 × g for 10 min to remove particles and aggregates.
4. Storage: If the sample is not tested immediately, please pack it in one batch, and store it at -20ÿ-70ÿ to avoid repeated freezing and thawing. try to avoid the use of hemolysis or Hyperlipidemia samples. If the serum contains a lot of particles, centrifuge or filter to remove it before testing; thaw at room temperature, do not store at 37°C or higher Heat to defrost.
5. Dilution: According to the actual situation, the specimen can be diluted by appropriate multiples (pre-experiment is recommended to determine the dilution ratio).'
Note: Normal mouse serum or plasma samples are recommended to be diluted 1:2.

Reagent preparation

1. Take the kit out of the refrigerator 30 minutes in advance and equilibrate to room temperature.
2. Washing buffer: The concentrated washing solution taken out of the refrigerator may have crystals, which is a normal phenomenon. After heating and shaking gently, the crystals are completely dissolved. Reconfigure. The concentrated washes were diluted with double distilled water (1:20). Unused ones were put back at 4°C.
3. Standard: add 1.0ml of standard/specimen diluent (1b) to the freeze-dried standard (1a), after it is completely dissolved, let stand for 15 minutes to mix (concentration: 8000pg/ml), and then dilute as needed, as shown in the figure below (the following concentrations are recommended for the standard curve: 8000, 4000, 2000, 1000, 500, 250, 125, 0 pg/ml). The diluted standard should not be reused, and the

unused standard should be repackaged according to the one-time dosage, and then placed in the Store at -20ÿ-70ÿ, single use, avoid repeated freezing and thawing.

Standard dilution method:




4. Biotinylated antibody working solution: Calculate the total amount according to the need of 100 ÿl per well, and prepare 100-200 ÿl more. Biotinylated Antibody Diluent (2b) Dilute the concentrated biotinylated antibody (2a) (1:100). Best to use now. (Refer to the table below for the dilution method)

| Number of slats used | Concentrated biotinylated antibodies | | Biotinylated Antibody Diluent |
|---|---|---|---|
| 12 | 110μL | + | 10890μL |
| 10 | 90μL | + | 8910μL |
| 8 | 70μL | + | 6930μL |
| 6 | 50μL | + | 4950μL |
| 4 | 33μL | + | 3267μL |
| 2 | 17μL | + | 1683μL |
| 1 | 9μL | + | 891μL |

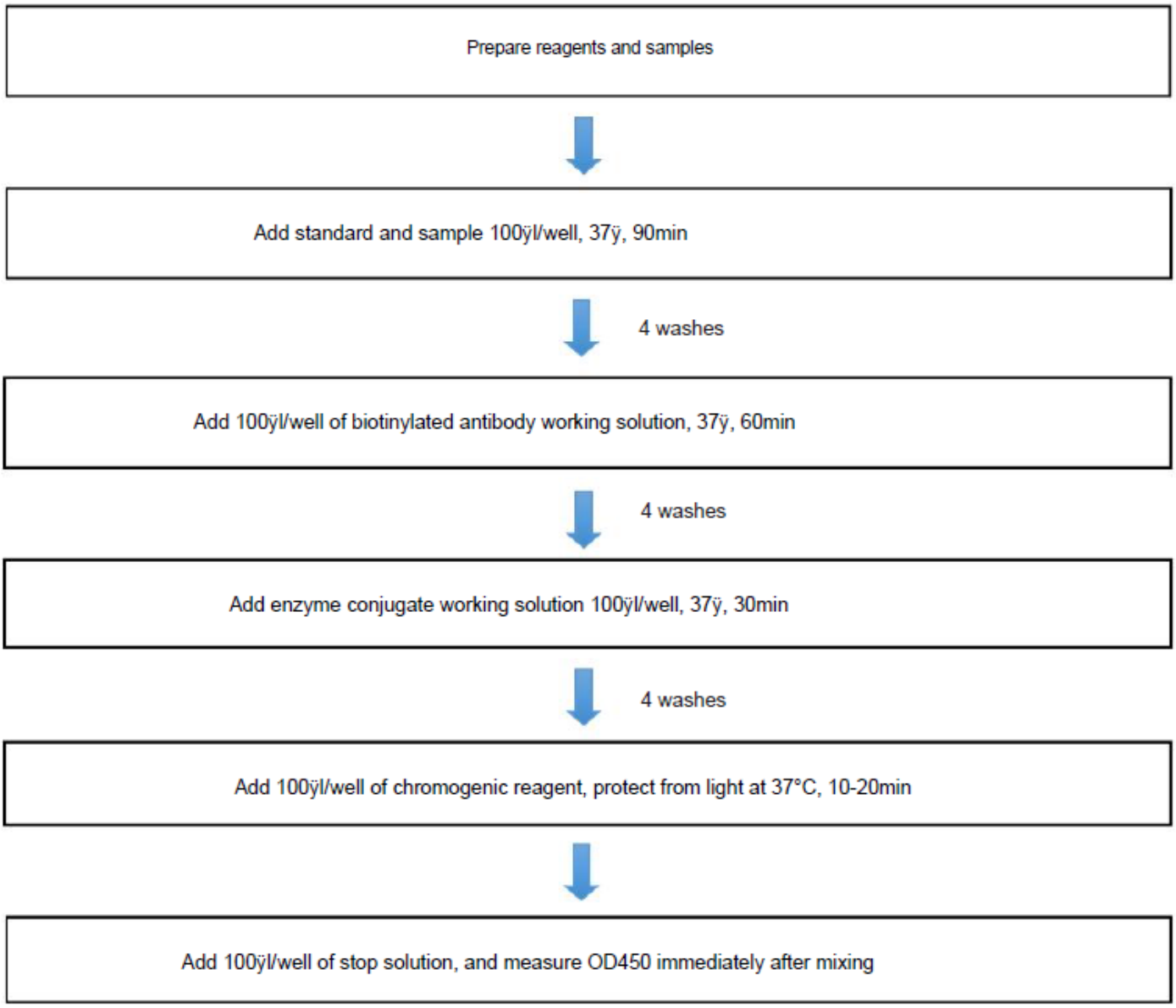
5. Enzyme Conjugate Working Solution: Dilute Concentrated Enzyme Conjugate (3a) with Enzyme Conjugate Diluent (3b) (1:100). Best to use now.
(Refer to the table below for the dilution method)

| 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 required number of strips according to the number of samples to be tested and the number of standards, and add 1 well as a blank control well. Samples and different concentrations were Standard (100 ÿl/well) was added to the corresponding well (only standard/sample dilution was added to zero well), the reaction well was sealed with sealing tape, and incubated in a 37°C incubator 90 minutes (except blank control wells).
3. Wash the plate 4 times: (1) Automatic plate washer: The required amount of washing solution to be injected is 350 ÿl, and the interval between injection and aspiration is 15-30 seconds. (2) Manual plate washing: throw out all the holes For liquid, add 350 ÿl of washing solution to each well, shake off the liquid after standing for 30 seconds, and pat dry on a thick layer of absorbent paper.
4. Add biotinylated antibody working solution (100 ÿl/well). Seal the reaction wells with sealing tape and incubate at 37°C for 60 minutes (except for the blank control wells).
5. Wash the plate 4 times.
6. Add enzyme conjugate working solution (100 ÿl/well). Seal the reaction wells with sealing tape and incubate at 37°C for 30 minutes (except for the blank control wells).
7. Wash the plate 4 times.
8. Add 100ÿl/well of chromogenic reagent, protect from light, and incubate at 37°C for 10-20 minutes.
9. Add 100 ÿl/well of stop solution, and measure the OD450 value immediately after mixing (within 5 minutes).

Operation flow chart




Operation point tips

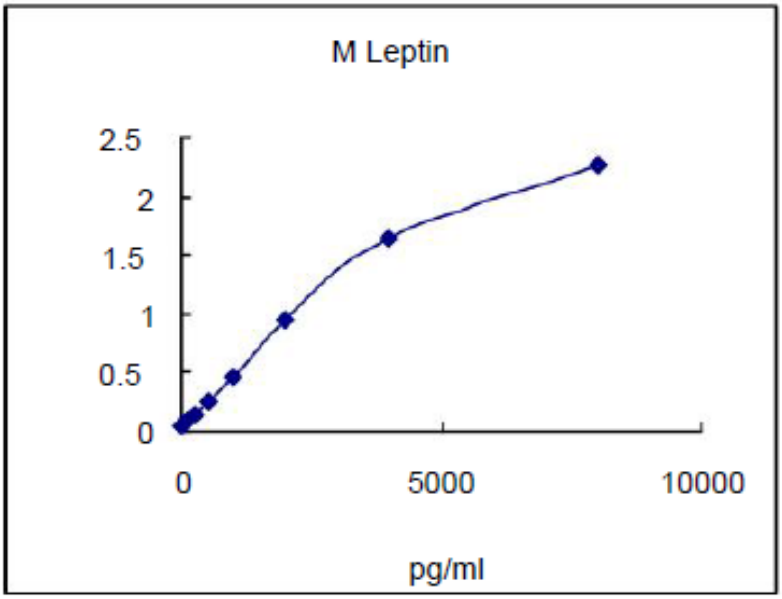
1. Mix thoroughly when preparing various reagents, but avoid generating a lot of bubbles, so as to avoid adding a lot of bubbles when adding samples, resulting in sample addition errors.
2. To avoid cross-contamination, remember to replace the tips in time when adding different concentrations of standards, different samples, and different reagents.
3. To ensure accurate results, seal the wells with new sealing tape before each incubation. 4. Before adding the developer, it should remain colorless, and do not use the developer solution that has turned blue. The optimal color development time is important for the standard curve, meat It can be seen that the first 3-4 wells have gradient blue, the latter 3-4 wells have no obvious difference, and the zero well can be

terminated without blue. 5. A standard curve should be made for each test. According to the content of the factor to be tested in the sample, the sample should be appropriately diluted or concentrated, and it is best to do a pre-experiment.

result judgment

1. The OD value of each standard and sample should be subtracted from the OD value of blank wells. If duplicate wells are used, the average value should be calculated.
2. Use computer software to take 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 diluted properly and then re-measured. When calculating the concentration, multiply the dilution ratio to calculate the content of the sample.
4. Reference data:

| Standard concentration (pg/ml) | OD value 1 | OD value 2 | average value | Correction value |
|---|---|---|---|---|
| 0 | 0.035 | 0.037 | 0.036 | —— |
| 125 | 0.096 | 0.089 | 0.093 | 0.057 |
| 250 | 0.140 | 0.146 | 0.143 | 0.107 |
| 500 | 0.253 | 0.256 | 0.255 | 0.219 |
| 1000 | 0.464 | 0.458 | 0.461 | 0.425 |
| 2000 | 0.940 | 0.943 | 0.942 | 0.906 |
| 4000 | 1.650 | 1.658 | 1.654 | 1.618 |
| 8000 | 2.258 | 2.256 | 2.257 | 2.221 |



This picture is for reference only, the standard curve drawn by the standard product in the same test shall prevail

Reproducibility of results

The coefficients of variation between plates and within plates were all <10%.

Sensitivity

The lowest dose of Leptin detected in mice was less than 62pg/ml. Minimum detection method: the average OD value of 20 zero standards is increased by two standard deviations,
Then calculate the corresponding concentration.

Specificity

This kit can detect natural and recombinant mouse Leptin, and do specific tests in parallel at 50ng/ml, and neither of them reacts with the following cytokines and proteins.

| recombinant human cytokine | recombinant mouse cytokine | native protein |
|---|---|---|
| ANG | GM-CSF | bovine FGF acidic |
| AR | IL-1ÿ | bovine FGF basic |
| CNTF | IL-1ÿ | human PDGF |
| ÿ-ECGF | IL-3 | porcine PDGF |
| EGF | IL-4 | human TGF-ÿ1 |
| Epo | IL-5 | |
| FGF acidic | IL-5Rÿ | |
| FGF basic | IL-6 | |
| FGF-4 | IL-7 | |
| FGF-5 | IL-9 | |
| FGF-6 | IL-10 | |
| G-CSF | IL-13 | |
| GM-CSF | Leptin | |
| sgp130 | LIF | |
| GROÿ | MIP-1ÿ | |
| GROÿ | MIP-1ÿ | |
| GROÿ | SCF | |
| HB-EGF | TNF-ÿ | |
| HGF | | |
| IFN-ÿ | | |

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