Human Leptin AExSet® antibody pair for ELISA

Instructions for Use | Cat. No.: ASEH0056

1. Content

Component	CAT	Volume
CA(capture antibody 100X)	DSEH005605/15CA	1 vial
S(Standard)	DSEH0056S	1 vial
DA(Detect Antibody 100X)	DSEH005605/15DA	1 vial
SH(Streptavidin-HRP 100X)	DSSH0105/15	1 vial

Intended Use

For the development of sandwich ELISAs to measure natural and recombinant Human Leptin. The Reagent Diluent recommended may be suitable for most cell culture supernate, serum, and plasma samples. The Reagent Diluent selected for use can alter the performance of an immunoassay. Reagent Diluent optimization for samples with complex matrices such as serum and plasma, may improve their performance in this assay.

The reagents are prepared as described in this package insert.

The recommended microplates, buffers, diluents, substrates, and solutions are used.

2. Reagent Preparation

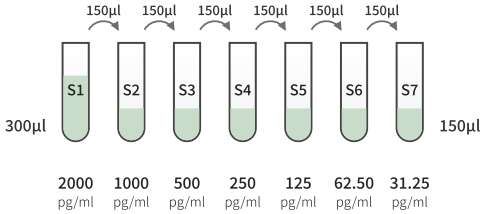
1X CA Preparation: Make a 1:100 dilution of the concentrated capture Antibody solution with carrier protein-free PBS, mix well and prepare for use.

1X DA Preparation: Make a 1:100 dilution of the concentrated Detect Antibody solution with **AB**, mix well and prepare for use.

1X SH Preparation: Make a 1:100 dilution of the concentrated Streptavidin-HRP solution with **AB**, mix well and prepare for use.

Standard Curve Preparation: Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization (concentration of reconstituted standard = 20000 pg/ml). Allow the standard to reconstitute for at least 15 minutes. Mix well prior to making dilutions.

The Human Leptin Standard DSEH0056S 20000 pg/ml 30 µl + 270 µl **AB** serves as the high standard (2000 pg/ml). Pipette 150 µl of **AB** into each tube. Use the high standard to produce a 1:1 dilution series. Mix each tube thoroughly before the next transfer. **AB** serves as the zero standard (0 pg/ml).



3. Assay Procedure

Plate Preparation

1. Dilute the Capture Antibody to the working concentration in PBS without carrier protein. Immediately coat a 96-well microplate with 100 µl per well of the diluted Capture Antibody(1X **CA**). Seal the plate and incubate overnight at 4°C.

2. Discard the liquid in the well, inverting the plate and blotting it against clean paper towels.

3. Block plates by adding 250 µl of Blocking Buffer to each well. Incubate at room temperature for a minimum of 2 hours.

4. Repeat the aspiration/ wash as in step 2.

Assay Procedure

Bring all reagents to room temperature before use. Allow to stand for at least 15 minutes after the standard proteins have been dissolved. Other working solutions should be used as they are prepared.

1. Prepare all reagents and working standards as directed in the previous sections.

2. Remove excess **CP** (Coated Plate) strips from the plate frame, return them to the foil pouch and reseal.

3. Add 50 µl of **AB** (Assay Buffer) to each well.

4. Add 50 µl of Standard or sample per well. Ensure reagent addition is uninterrupted and completed within 15 minutes.

5. Add 50 µl of **DA** 1X (Detect Antibody) to each well.

6. Cover with an **SF** (Sealer Film). Incubate at room temperature (18 to 25°C) for 1 hour on a microplate shaker set at 500 rpm.

7. Aspirate each well and wash, repeating the process four times. Wash by filling each well with **WB** (Washing Buffer 300 µl). Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining **WB** (Washing Buffer) by aspirating or decanting. Invert the plate and blot it against clean paper towels.

8. Add 100 µl of **SH** 1X (Streptavidin-HRP) to each well.

9. Cover with a new **SF** (Sealer Film). Incubate at room temperature (18 to 25°C) for 30 min on a microplate shaker set at 500 rpm.

10. Repeat aspiration/wash as in step 7.

11. Add 100 µl of **TS** (TMB Substrate) to each well. Incubate for 5-30 minutes at room temperature.

12. Add 100 µl of **SS** (Stop Solution) to each well.

13. Determine the optical density within 30 minutes, using microplate reader set to 450 nm corrected with 570 nm or 630 nm.

4. Other Materials & Solutions Required

AExSet® Ancillary Reagent Kit (5 plates): containing 96 well microplates, plate sealers, substrate solution, stop solution, plate coating buffer (PBS), wash buffer, and assay buffer.

The components listed above may be purchased separately:

96 well microplates: AREX Biosciences, Catalog # DSEP01.

Plate Sealers: AREX Biosciences, Catalog # DSSF01.

Coating Buffer: 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.5 mM KH_2PO_4, pH 7.2-7.4, 0.2 µm filtered. AREX Biosciences, Catalog # DSCB01.

Blocking Buffer: AREX Biosciences, Catalog # DSBB01.

Wash Buffer: 0.05% Tween 20 in PBS, pH 7.2-7.4. AREX Biosciences, Catalog # DSWB01.

Assay Buffer: 0.5%BSA，0.05%Tween20，PBS Solution.AREX Biosciences, Catalog # DSAB01

Substrate Solution: Tetramethylbenzidine. AREX Biosciences, Catalog # DSTS01.

Stop Solution: 0.5mol/L H_2SO_4. AREX Biosciences, Catalog # DSSS01.

Precautions

The Stop Solution suggested for use with this kit is an acid solution.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

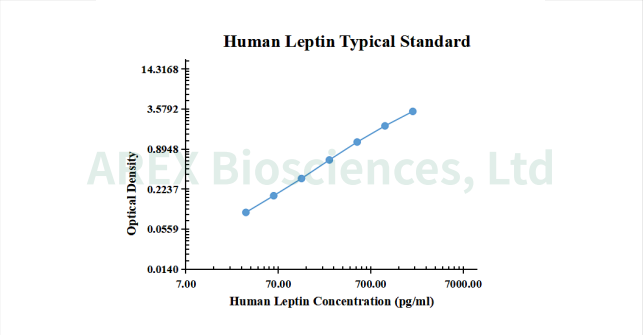
5. Calculation of Results

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the Human Leptin concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

6. Typical Data



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