

**KSA084Bo01 96T x 10**

**ELISA Kit DIY Materials**

**For Leptin (LEP)**

**Organism Species: Bos taurus; Bovine (Cattle)**

**Instruction manual**

FOR IN VITRO AND RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1th Edition

## **[ INTENDED USE ]**

For the development of sandwich ELISA to measure LEP in bovine serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids in vitro. This kit contains sufficient materials for preparation of at least ten 96-well plates.

## **[ REAGENTS AND MATERIALS PROVIDED ]**

**Capture Antibody:** 1 vial

**Detection Antibody:** 1 vial

**Standard:** 10 vials

**Streptavidin-HRP:** 1 vial

**TMB Substrate:** 2 vials

**96-well Plate:** 2x5 plates

## **[ MATERIALS REQUIRED BUT NOT SUPPLIED ]**

Assay Kit DIY Support Pack 1

**Notes:** The recommended Cloud-Clone's products of diluents and buffers are validated in the lab, other reagents selected for use can alter the performance of an immunoassay.

## [ STORAGE ]

Antibodies, Standard and Streptavidin-HRP should be stored at -20°C. TMB should be stored at 4°C. 96-well Plate could be stored at room temperature. The reagents are valid for 12 months, they are stable for one month after opening when stored at 4°C. Please make all solutions fresh before the experiment.

## [ REAGENT PREPARATION ]

Bring all components to room temperature (18-25°C) before use. Working solutions should be prepared and used immediately.

**Standard:** Reconstitute one vial of Standard with 1.0mL of working solution of Reagent Diluent 1, kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard in the stock solution is 80ng/mL. Please firstly dilute the stock solution to 20ng/mL and the diluted standard serves as the highest standard (20ng/mL). Then make serial dilution of the Standard with working solution of Reagent Diluent 1 in 2 times to gain a proper standard curve.

**Capture Antibody:** Briefly spin or centrifuge the stock Capture Antibody before use. Aspirate appropriate volume of Capture Antibody, 1: 500 dilute in working solution of Coating Buffer for plate coating.

**Detection Antibody:** Briefly spin or centrifuge the stock Detection Antibody before use. Aspirate appropriate volume of Detection Antibody, 1: 1000 dilute in working solution of Reagent Diluent 2.

**Streptavidin-HRP:** Briefly spin or centrifuge the stock Streptavidin-HRP before use. Aspirate appropriate volume of the reagent, 1: 100 dilute in working solution of Reagent Diluent 3.

Cloud-Clone's product of Assay Kit DIY Support Pack 1 (Catalog: IS049), which includes all kinds of buffers is high recommended for reagent preparation.

## **[ ASSAY PROTOCOL ]**

### **Plate Preparation:**

1. Dilute the Capture Antibody to working concentration in Coating Buffer. Immediately coat the 96-well microplates with 100µL per well of the diluted Capture Antibody. Seal the plate and incubate overnight at 4°C or incubate at 37°C for 2 hours.
2. Aspirate the solution and wash with 350µL of working solution of Wash Buffer to each well using a squirt bottle, multi-channel pipette, manifold dispenser or auto-washer, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper.
3. Block plates by adding 200µL of working solution of Blocking Buffer to each well. Incubate at 37°C for 1.5 hours.
4. Repeat the aspiration/wash process as in step 2. The plates are now ready for sample detection.

### **Common Used Assay Procedure:**

1. Add 100µL of different concentration of standards, samples and diluent into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.
2. Repeat the aspiration/wash process as in step 2 of plate preparation.
3. Add 100µL of Detection Antibody working solution to each well, cover the wells, and incubate for 1 hour at 37°C.
4. Repeat the aspiration/wash process for 3 times as in step 2.
5. Add 100µL of working solution of Streptavidin-HRP to each well, cover the wells, and incubate for 30 minutes at 37°C.
6. Repeat the aspiration/wash process for total 5 times as in step 2.
7. Add 90µL of TMB Substrate to each well. Cover the wells, and incubate for 10 - 20 minutes at 37°C. Protect from light.
8. Add 50µL of Stop Solution (1mol/L H<sub>2</sub>SO<sub>4</sub>) to each well. Mix the liquid by tapping the side of the plate.
9. Run the microplate reader and conduct measurement at 450nm immediately.

## **[ DETECTION RANGE FOR REFERENCE ]**

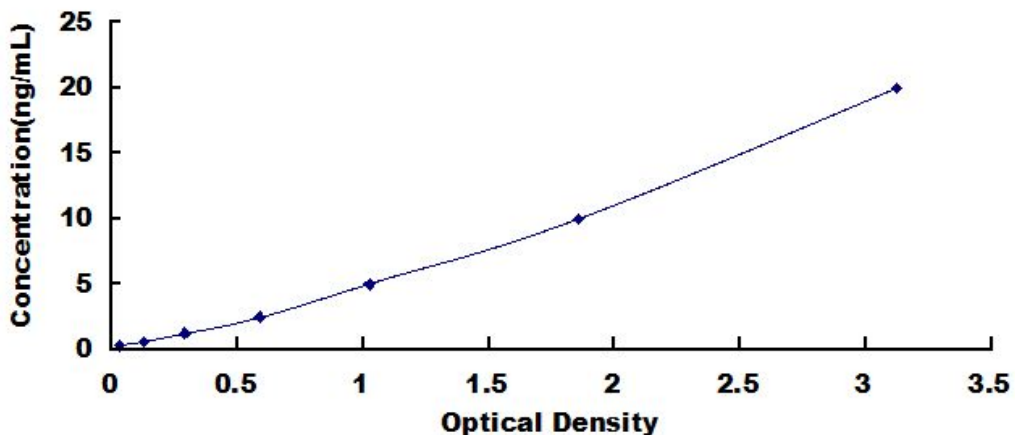
The detection range of ELISA prepared by these materials in our lab is 0.312-20ng/mL.

## **[ SPECIFICITY ]**

The Antibody Pairs in the kit have high sensitivity and excellent specificity for detection of LEP. No significant cross-reactivity or interference between LEP and analogues was observed.

## **[ TYPICAL DATA ]**

Typical standard curve below is provided for reference only. A standard curve should be generated from each set of experiment.



Typical Standard Curve of ELISA kit for Bovine, LEP.