KSA991Hu11 96T x5
ELISA Kit DIY Materials
For Ghrelin (GHRL)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR IN VITRO AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition

[INTENDED USE]

For the development of competitive inhibition ELISA to measure GHRL in human serum, plasma and other biological fluids in vitro. This kit contains sufficient materials for preparation of at least five 96-well plates.

[REAGENTS AND MATERIALS PROVIDED]

Capture Antigen: 1 vial

Biontin-labeled Competitor: 1 vial

Standard: 5 vials

Streptavidin-HRP: 1 vial

TMB Substrate: 1 vial

96-well Plate: 5 plates

[MATERIALS REQUIRED BUT NOT SUPPLIED]

Assay Kit DIY Support Pack 2

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]

Antigens, Standard, Biontin-labeled Competitor 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 is 30,000pg/mL. Then make serial dilution of the Standard with working solution of Reagent Diluent 1 in 3 times to gain a proper standard curve.

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

Biontin-labeled Competitor: Briefly spin or centrifuge the stock Biontin-labeled Competitor before use. Aspirate appropriate volume of Biontin-labeled Competitor, 1: 1,000 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 2 (Catalog: IS050), which includes all kinds of buffers is high recommended for reagent preparation.

[ASSAY PROTOCOL]

Plate Preparation:

1. Dilute the Capture Antigen to working concentration in Coating Buffer. Immediately coat the 96-well microplates with 100μL per well of the diluted Capture Antigen. 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 50μL of different concentrations of standards, samples and diluent into the appropriate wells. And then add 50μL of working solution of Biontin-labeled Competitor to each well immediately. Shake the plate gently (using a microplate shaker is recommended). Cover with the Plate sealer. Incubate for 1 hour at 37°C.
2. Repeat the aspiration/wash process for 3 times as in step 2 of plate preparation.
3. Add 100μL of working solution of Streptavidin-HRP to each well, cover the wells, and incubate for 30 minutes at 37°C.
4. Repeat the aspiration/wash process for total 5 times as in step 2.
5. Add 90μL of TMB Substrate to each well. Cover the wells, and incubate for 10 - 20 minutes at 37°C. Protect from light.
6. Add 50μL of Stop Solution (1mol/L H_2SO_4) to each well. Mix the liquid by tapping the side of the plate.
7. Run the microplate reader and conduct measurement at 450nm immediately.

[DETECTION RANGE FOR REFERENCE]

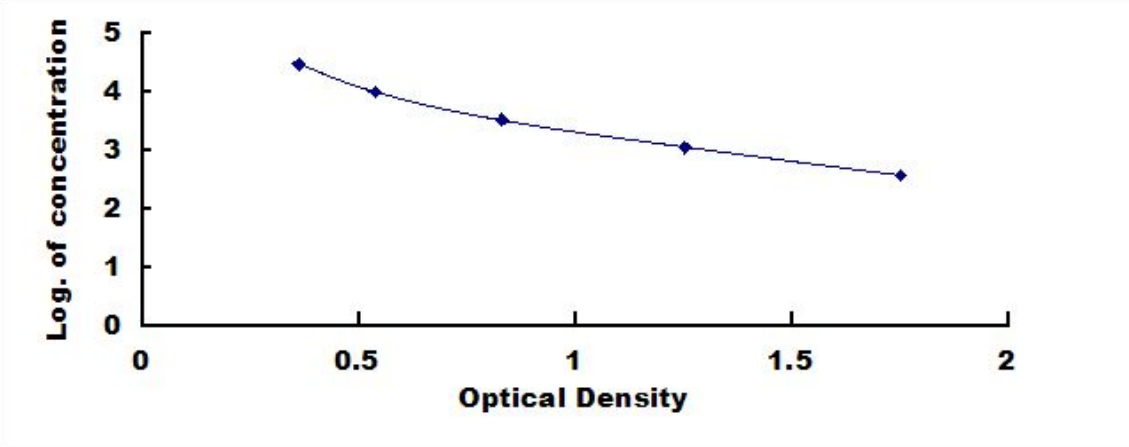
The detection range of ELISA prepared by these materials in our lab is 370.4-30,000pg/mL.

[SPECIFICITY]

This assay has high sensitivity and excellent specificity for detection of GHRL. No significant cross-reactivity or interference between GHRL 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 Human, GHRL.