# KSB995Hu01

**Product Name:** ELISA Kit DIY Materials for Hemojuvelin (HJV)

**Organism Species:** Homo sapiens (Human)

**Contents:**

Capture Antibody to Hemojuvelin (HJV): 1 vial

Detection Antibody to Hemojuvelin (HJV):1 vial

Standard:2 vials

Streptavidin-HRP: 1 vial

TMB Substrate: 2 vials

96-well Plate: 10 plates

**Intended Use:**

For the development of sandwich ELISA to measure HJV in human 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.

**Other Reagents Required:**

ELISA/CLIA Support Pack 1. (Catalog IS049)

**Storage:**

Antibodies, Standard and Streptavidin-HRP should be stored at -20ºC. TMB should be stored at 4ºC. 96-well Plate could be stored at room temperature.

The reagents are valid for twelve months. Please make all solutions fresh before the experiment.

**Reagent Preparation:**

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

**Standard:** Reconstitute one vial of Standard with 1 mL 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 10,000pg/mL. Then make serial dilution of the Standard with Reagent Diluent 1 in 2 times to gain a proper standard curve.

**Capture Antibody:** Briefly spin or centrifuge the stock Capture Ab before use. Aspirate appropriate volume of Capture Antibody, 1: 1000dilute in Coating Buffer for plate coating.

**Detection Antibody:** Briefly spin or centrifuge the stock Detection Ab before use. Aspirate appropriate volume of Detection Antibody, 1: 2000 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.

**Assay Protocol:**

**Plate Preparation:**

1. Dilute the Capture Antibody to working concentration in Coating Buffer. Immediately coat the 96-well microplates with 100 µL 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 autowasher, 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 200 µL 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.

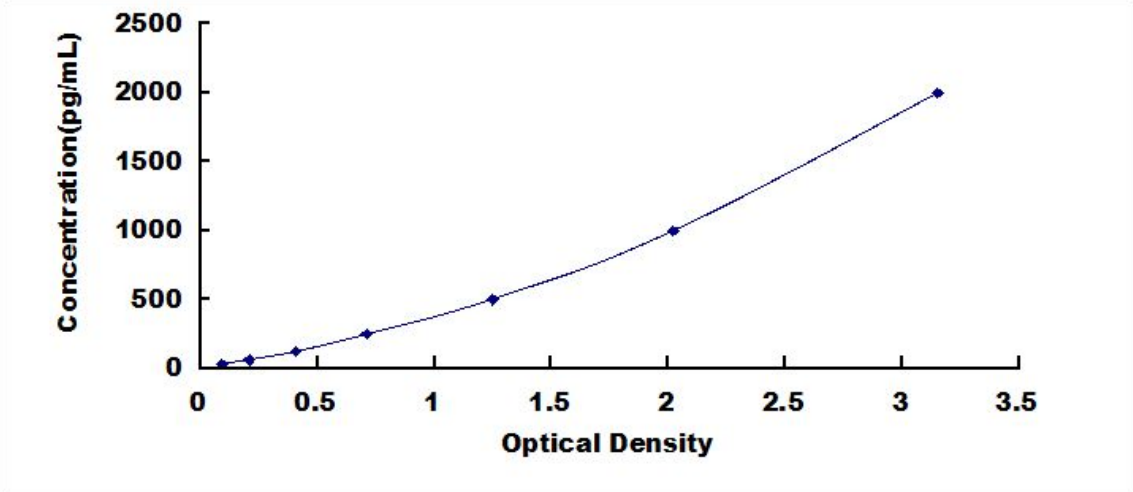
**Assay Procedure:**

1. Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100µL each of dilutions of standard, blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.
2. Remove the liquid of each well, don't wash.
3. Add 100µL of Detection Ab working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37°C.
4. 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 autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.
5. Add 100µL of working solution of Streptavidin-HRP to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37°C.
6. Repeat the aspiration/wash process for total 5 times as conducted in step 4.

7. Add 90μL of TMB Substrate to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of TMB Substrate.
8. Add 50μL of Stop Solution (1mol/L $H_2SO_4$) to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
9. Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

**Typical Standard Curve:**

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




**Typical Standard Curve for Hemojuvelin, Human ELISA.**

**Detectable Range:** The detection range of ELISA prepared by these materials in our lab is 31.2-2,000pg/mL..

**Specificity:** The Abs in the kit have high sensitivity and excellent specificity for detection of Hemojuvelin (HJV). No significant cross-reactivity or interference between Hemojuvelin (HJV) and analogues was observed.