

**KSA182Ge11 96T x5  
ELISA Kit DIY Materials  
For Hyaluronic Acid (HA)  
Organism: General  
*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 competitive inhibition ELISA to measure HA in 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 Antibody:** 1 vial

**Biotin-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 ]**

Antibodies, Standard, Biotin-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 2.0mL 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 400ng/mL. Then make serial dilution of the Standard with Reagent Diluent 1 in 3 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: 400 dilute in Coating Buffer for plate coating.

**Biotin-labeled Competitor:** Briefly spin or centrifuge the stock Detection Antibody before use. Aspirate appropriate volume of Detection Antibody, 1: 500 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 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 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 Biotin-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<sub>2</sub>SO<sub>4</sub>) 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 ]**

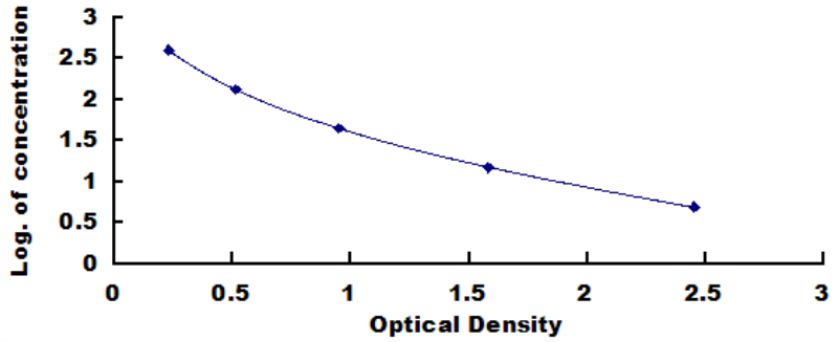
The detection range of ELISA prepared by these materials in our lab is 4.94-400ng/mL.

#### **[ SPECIFICITY ]**

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