

**KSH776Ra01 96T x 5**  
**ELISA Kit DIY Materials**  
**For Inter Alpha-Globulin Inhibitor H4 (ITIH4)**  
**Organism Species: *Rattus norvegicus* (Rat)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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2nd Edition

### **[ INTENDED USE ]**

This 'Do it Yourself (DIY)' assay kit contains materials for developing your own immunoassays. This may include the development of sandwich ELISA to measure ITIH4 in rat 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 five 96-well plates. Sample Preparation, Assay Protocol and Detection Range for Reference are provided as suggestions only. Researchers should optimize the use of kit materials and protocols for their own model system and determine if the kit is suitable for their samples and immunoassay.

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

| <b>Components</b>               | <b>Quantity</b> | <b>Form</b>                                |
|---------------------------------|-----------------|--|
| Standard                        | 5 ng            | Lyophilized, 5 vials                       |
| Capture Antibody                | 120ug/0.15 mL   | Liquid, 1 vial, contains 0.1% sodium azide |
| Biotinylated Detection Antibody | 20ug/0.06 mL    | Liquid, 1 vial, contains 0.1% sodium azide |
| Streptavidin-HRP                | 0.6 mL          | Liquid, 1 vial , contains 0.05% Proclin300 |

|               |          |                |
|---------------|----------|----------------|
| TMB Substrate | 48 mL    | Liquid, 1 vial |
| 96-well Plate | 96 wells | 5 plates       |

## **[ Recommended Buffers and Solutions ]**

ASSAY Kit DIY Support Pack 1, **Cat # IS049**, which includes Plate Sealer, Coating Buffer, Blocking Buffer, Reagent Diluent 1, Reagent Diluent 2, Reagent Diluent 3, Wash Buffer, Enhancer is highly recommended for reagent preparation.

**Notes:** The recommended Cloud-Clone's diluents and buffers contained in IS049 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 unopened 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 0.5mL of working solution of Reagent Diluent 1 (Cat # IS049), kept for 10 minutes at room temperature, shake gently (not to foam). The concentration of the standard is 10ng/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: 400 dilution in working solution of Coating Buffer (Cat # IS049) for plate coating.

**Detection Antibody:** Briefly spin or centrifuge the stock Detection Antibody before use. Aspirate appropriate volume of Detection Antibody, 1: 1000 dilution in working solution of Reagent Diluent 2 (Cat # IS049).

**Streptavidin-HRP:** Briefly spin or centrifuge the stock Streptavidin-HRP before use. Aspirate appropriate volume of the reagent, 1: 100 dilution in working solution of Reagent Diluent 3 (Cat # IS049).

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

## **[ SAMPLE PREPARATION ]**

Serum/plasma samples require about a 1,000 fold dilution. For example, to prepare a 1:1,000 dilution of sample, transfer 20 $\mu$ L of sample to 180 $\mu$ L PBS. This yields a 1:10 dilution. Next, dilute the 1:10 sample by transferring 10 $\mu$ L to 990 $\mu$ L PBS. You now have a 1:1,000 dilution of your sample. Mix thoroughly at each stage. Sample should be diluted by 0.01mol/L PBS(PH=7.0-7.2).

## **[ ASSAY PROTOCOL ]**

### **Plate Preparation:**

1. Dilute the Capture Antibody to working concentration in Coating Buffer (Cat # IS049). Immediately coat the 96-well microplates with 100 $\mu$ L 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 350 $\mu$ L of working solution of Wash Buffer (Cat # IS049) 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 200 $\mu$ L of working solution of Blocking Buffer (Cat # IS049) 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.

### **Commonly Used Assay Procedure:**

1. Add 100 $\mu$ L of different concentration of standards, samples 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.dd 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 (Cat # IS049) 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 ]**

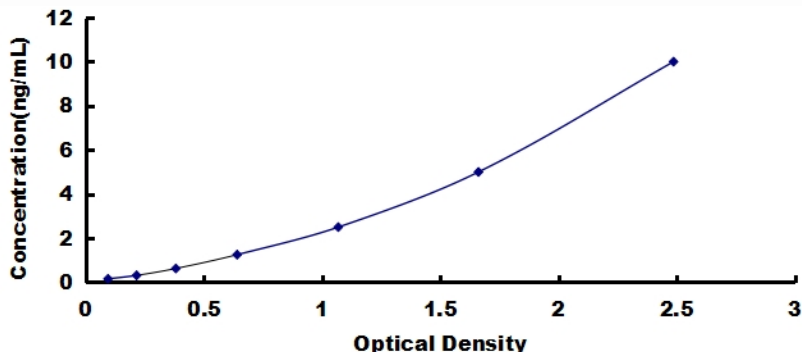
The detection range of ELISA prepared by these materials in our lab is 0.156-10ng/mL.

### **[ SPECIFICITY ]**

The Antibody Pairs in the kit have high sensitivity and excellent specificity for detection of ITIH4.

### **[ TYPICAL DATA ]**

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



**Typical Standard Curve of ELISA Assay for Rat, ITIH4.**