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User's Manual

Human Interleukin 1 Receptor-Like 1, IL1RL1 ELISA Kit

| REF | **CKERS-IL1RL1-257H** |

Σ **5 plates**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

PRODUCT INFORMATION

Intended Use

The human IL1RL1 ELISA kit is for the quantitative determination of human IL1RL1.

This ELISA kit contains the basic components required for the development of sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on five 96-well plates.

Principles of Testing

The ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for IL1RL1 coated on a 96-well plate. Standards and samples are added to the wells, and any IL1RL1 present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-IL1RL1 monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produce color in proportion to the amount of IL1RL1 present in the sample streptavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450nm.

Storage

Detection Antibody should be protected from prolonged exposure to light. Aliquot all other reagents and store at -20°C to -70°C in a manual defrost freezer.

Reconstitution And Storage

Bring all reagents to room temperature before use.

Capture Antibody: 1.0 mg/ml of mouse anti-IL1RL1 monoclonal antibody. Dilute to a working concentration of 2.0µg/ml in CBS before coating.

Detection Antibody: 0.5 mg/ml of rabbit anti-IL1RL1 monoclonal antibody conjugated to horseradish-peroxidase(HRP). Dilute to a working concentration of 0.5 µg/ml in detection antibody dilution buffer before use.

Standard: Each vial contains 55ng of recombinant IL1RL1. Reconstitute with 1ml detection antibody dilution buffer. After reconstitution, store at -20°C to -70°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 800 pg/ml is recommended.

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100uL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.

2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.

3. Block plates by adding 300µl of blocking buffer to each well. Incubate at room temperature for a minimum of 1hour.

4.   Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1.   Add 100μl of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.

2.   Repeat the aspiration/wash as in step 2 of plate preparation.

3.   Add 100μl of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.

4.   Repeat the aspiration/wash as in step 2 of plate preparation.

5.   Add 200μl of substrate solution to each well. Incubate for 20 minutes at room temperature **(if substrate solution is not as requested, the incubation time should be optimized)**. Avoid placing the plate in direct light.

6.   Add 50μl of stop solution to each well. Gently tap the plate to ensure thorough mixing.

7.   Determine the optical density of each well immediately, using a microplate reader set to 450nm.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.

Construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.

To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.
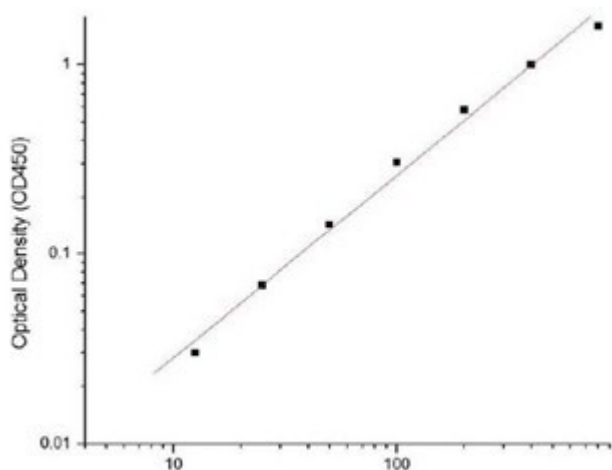
IL1RL1 concentration(pg/ml)

Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
12.5	0.030
25	0.068
50	0.142
100	0.306
200	0.577
400	0.997
800	1.604

Sensitivity

12.5 pg/mL

Precautions

The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.