Version 07-02/25

User's Manual

Human Interleukin 13 Receptor, Alpha 1, IL13RA1 ELISA Kit

REF **CKERS-IL13RA1-255H**

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.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The human IL13RA1 ELISA kit is for the quantitative determination of human IL13RA1.

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 IL13RA1 coated on a 96-well plate. Standards and samples are added to the wells, and any IL13RA1 present binds to the immobilized antibody. The wells are washed and a biotinylated rabbit anti-IL13RA1 monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich". To produce color in proportion to the amount of IL13RA1 present in the sample streptavidin-HRP and TMB substrate solution are loaded. The absorbances of the microwell are read at 450nm.

Reagents And Materials Provided

1. **Bring all reagents to room temperature before use.**
2. **Capture Antibody:** 0.5 mg/mL of mouse anti-IL13RA1 monoclonal antibody. Dilute to a working concentration of 2.0 µg/mL in CBS before coating.
3. **Detection Antibody:** 0.5 mg/mL of #rabbit anti-IL13RA1 monoclonal antibody conjugated to horseradish-peroxidase(HRP). Dilute to a working concentration of 1.0 µg/mL in detection antibody dilution buffer before use.
4. **Standard:** Each vial contains 80 ng of recombinant IL13RA1. Reconstitute with 1 mL 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 2,000 pg/mL is recommended.

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.

Plate Preparation

1. Dilute the capture antibody to the working concentration in CBS. Immediately coat a 96-well microplate with 100 µL 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.

Reagent Preparation

CBS: 0.05M Na2CO3, 0.05M NaHCO3, pH9.6, 0.2μm filtered

TBS: 25mM Tris, adjust pH to 7.4 by HCl

Wash Buffer: 0.05% Tween20 in TBS, pH 7.2-7.4

Blocking Buffer: 2% BSA in Wash Buffer

Sample dilution buffer: 0.1% BSA in wash buffer, pH7.2-7.4, 0.2μm filtered

Detection antibody dilution buffer: 0.5% BSA in wash buffer, pH 7.2-7.4, 0.2μm filtered.

Substrate Solution: To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution: 10 mg/mL TMB in DMSO

Substrate dilution buffer: 0.05M Na2HPO4 and 0.025M citric acid; adjust pH to 5.5

Substrate working solution: For each plate dilute 250 μL substrate stock solution in 25ml substrate dilution buffer and then add 80μL 0.75% H2O2, mix it well

Stop Solution: 2N H2SO4

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

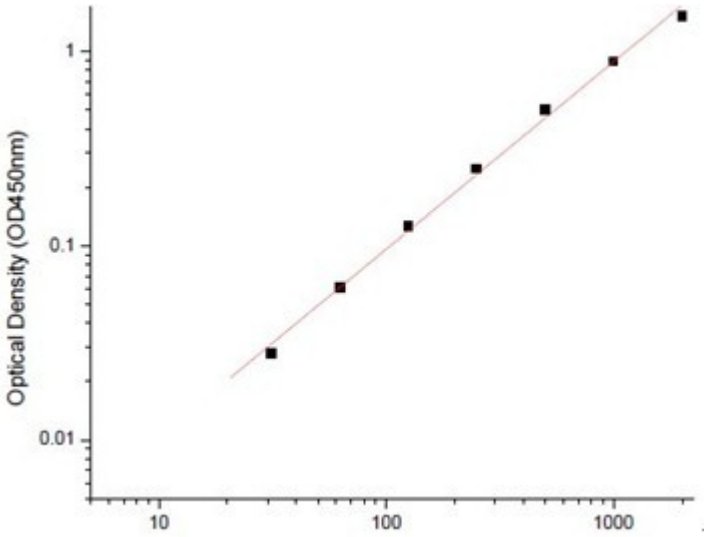
Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.

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.



IL13RA1 concentration(pg/ml)

Concentration (pg/ml)	Zero standard subtracted OD
0	0.000
31.25	0.028
62.5	0.061
125	0.126
250	0.250
500	0.502
1000	0.892
2000	1.519

Sensitivity

The minimum detectable dose of human IL13RA1 was determined to be approximately 31.25 pg/mL. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

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.