IL13RA1 (Human) ELISA Kit

Catalog Number KA2212

96 assays

Version: 01

Intended for research use only

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Introduction

Background

IL13RA1 encodes the subunit of the interleukin 13 receptor. This subunit forms a receptor complex with IL4 receptor alpha, a subunit shared by IL13 and IL4 receptors. In addition, it serves as a primary IL13-binding subunit of the IL13 receptor, and may also be a component of IL4 receptors. IL13RA1 has been shown to bind tyrosine kinase TYK2, and thus may mediate the signaling processes that lead to the activation of JAK1, STAT3 and STAT6 induced by IL13 and IL4.

IL13RA1 binds with low affinity to interleukin-13 (IL-13). Together with IL4R, IL13RA1 can form a functional receptor for IL-13. It also serves as an alternate accessory protein to the common cytokine receptor gamma chain for interleukin-4 (IL4) signaling, but cannot replace the function of IL2RG in allowing enhanced interleukin-2 (IL-2) binding activity. Interleukin-13 receptor is a complex of IL4R, IL13RA1, and possibly other components. It interacts with TRAF3IP1 and its highest levels are found in heart, liver, skeletal muscle and ovary while the lowest levels are found in brain, lung and kidney. IL13RA1 is also found in B-cells, T-cells and endothelial cells. Studies have shown that the WSXWS motif appears to be necessary for proper protein folding and thereby efficient intracellular transport and cell-surface receptor binding while the box 1 motif is required for JAK interaction and/or activation.

Principle of the Assay

The IL13RA1 (Human) ELISA Kit contains the components necessary for quantitative determination of natural or recombinant hIL-13 Rα1 concentrations within any experimental sample including cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a “Sandwich” Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a “sandwich” format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator. The capture antibodies coated to the bottom of each well are monoclonal, specific for a particular epitope on the Human IL-13 Rα1 cytokine while the user-added detection antibodies are polyclonal for binding to a variety of epitopes on the bound target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase. After incubation and “sandwiching” of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a colorimetric reaction to ensue upon substrate addition. When the substrate TMB (3, 3’, 5, 5’-Tetramethylbenzidine) is added, the reaction catalyzed by peroxidase yields a blue color that is representative of the antigen concentration. Upon sufficient color development, the reaction can be terminated through addition of Stop Solution (2 M Sulfuric Acid) where the color of the solution will turn yellow. The absorbance of each well can then be read by a spectrophotometer, allowing for generation of a standard curve and subsequent determination of protein concentration.

General Information

Materials Supplied

List of component

Component	State	Amount
96-Well Microplate or Strips Coated w/ Capture Antibody	ready to use	1
Biotin-Conjugated Detection Antibody	Reconstitute with 67 µl H_2O Dilute Using Detection Antibody Diluent	Lyophilized (1.2 µg)
Avidin-HRP Conjugate	ready to use	10 µl
Cytokine Protein Standard	Reconstitute with 100µl H_2O Dilute Using Standard Diluent	Lyophilized (30 ng)
Ready-to-Use Substrate	ready to use	11 ml
Stop Solution	ready to use	11 ml
Adhesive Plate Sealers	ready to use	4 Sheets
Wash Buffer (10X)	Dilute to 1X Using Pure H_2O	50 ml
Protein Standard Diluent	ready to use	15 ml
Detection Antibody Diluent	ready to use	15 ml
HRP Diluent	ready to use	15 ml

Storage Instruction

If used frequently, reagents may be stored at 2-8℃. If used infrequently, reagents should be stored at -20℃.

Materials Required but Not Supplied

- ✓ Microplate reader able to measure absorbance at 450 nm (with correction wavelength set to 540 nm or 570 nm)
- ✓ Micropipettes with capability of measuring volumes ranging from 1 µl to 1 ml
- ✓ Deionized or sterile water
- ✓ Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer
- ✓ Graph paper or computer software capable of generating or displaying logarithmic functions
- ✓ Absorbent paper or vacuum aspirator
- ✓ Test tubes or microfuge tubes capable of storing ≥1 ml
- ✓ Bench-top centrifuge (optional)
- ✓ Bench-top vortex (optional)
- ✓ Orbital shaker (optional)

Precautions for Use

- Assay Restrictions

✓ This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.

✓ Materials included in this kit should NOT be used past the expiration date on the kit label.

✓ Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.

✓ Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.

✓ The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.

- Health and safety Precautions

✓ Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.

✓ Stop Solution contains 2 M Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

Assay Protocol

Reagent Preparation

✓ All reagents should be diluted immediately prior to use.

Assay Procedure

✓ If possible, all incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells. Also, all provided solutions should be at ambient temperature prior to use.

✓ Avoid adding solutions into wells at an angle, always keep pipette tip perpendicular to plate bottom.

- Addition of Known Standard and Unknown Sample to Immunoassay

  Prior to applying an unknown sample to the Sandwich ELISA, the immunoassay must be performed using a serial dilution of a known standard sample in order to determine the standard curve. This is necessary to allow for the interpretation of results generated by the unknown samples.

1. Dilute the known standard sample from 16 ng/ml to 0 ng/ml in a series of microfuge tubes. Mix each tube thoroughly by inverting several times or by vortexing lightly to ensure proper equilibration. Add 100 µl of each serial dilution step into the wells of a specified row or column of the 96-well microtiter plate in duplicate or triplicate and incubate at room temperature for 2 hours. Seal the microplate air-tight using one of the microplate adhesive seals provided in this kit or Parafilm if readily available. *Note: If a standard curve has already been generated, substitute the standard with the unknown sample of interest.*

- Application of Detection Antibody to Capture Antibody-Bound Samples

1. Aspirate the protein standard solution out of the microplate wells. If your lab does not have a vacuum-based aspirator, you may dump the solutions from the microplate into a waste container and blot 3-4 times on a stack of paper towels until most or all of the liquid is removed from the wells. Dilute the 10X wash buffer to 1X using pure H2O. Add 300-400 µl of Wash Buffer to each well being used and gently shake for 5-7 minutes on an orbital shaker. Perform this wash step 4 times consecutively.
2. After the 4th wash step, dilute the detection antibody solution 1:180 in detection antibody diluent to a concentration of 100 ng/ml. Mix the test tube either by inverting several times or vortexing to ensure proper equilibration. Ensure that there is enough detection antibody solution for all wells being used. Add 100 µl of the diluted detection antibody solution into each well, seal the plate and incubate at room temperature for 2 hours.

- Conjugation of Avidin-Horseradish Peroxidase Enzyme with Detection Antibody

1. Remove the detection antibody solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Perform 4 consecutive wash steps with gentle shaking between each wash.
2. After the 4th wash step, dilute 5.5 µl of the Avidin-HRP conjugate 1:2000 in diluent for a total volume of 11

ml. Mix the test tube either by inverting several times or vortexing to ensure proper equilibration. Add 100 µl of diluted Avidin-HRP conjugate solution into each well and incubate at room temperature for 30 minutes.

- Application of Liquid Substrate for Colorimetric Reaction

1. Remove the Avidin-HRP conjugate solution out of the microplate wells by either vacuum-based aspirator or paper towel blotting. Prepare the TMB substrate solution by bringing it to room temperature without exposure to fluorescent or UV light as these may degrade the TMB. Perform 4 consecutive wash steps with gentle shaking between each wash.
2. After the 4th wash step, add 100 µl of TMB substrate solution into each well and incubate at room temperature for color development. The microplate should be kept out of direct light by either covering with an opaque object or putting it into a dark room. Closely monitor the color development as some wells may turn blue very quickly depending on analyte and/or detection antibody-HRP concentrations. Once the blue color has ceased to develop further, immediately add 100 µl of Stop Solution to each well being used. The color in the wells should immediately change from blue to yellow.
3. The microplate is now ready to be read by a microplate reader. Within 30 minutes of adding the Stop Solution, determine the optical density (absorbance) of each well by reading the plate with the microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. Caution: Readings made directly at 450 nm without correction may be higher and less accurate.

Data Analysis

Calculation of Results

1. Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard optical density.
2. Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4-Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis). Note: Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or “trend-line” through the plotted points via regression analysis. Note: Shown on the next page is an example of typical data produced by analysis of the standard sample.

The data and subsequent graph was obtained after performing a cytokine ELISA for Human IL-13 Rα1. Each known sample concentration was assayed in triplicate.

Human IL-13 Rα1 Standard Curve	
Concentration (pg/ml)	Average OD 450nm
16000	2.2
8000	1.75
4000	1.2
2000	0.75
1000	0.45
500	0.25
250	0.14

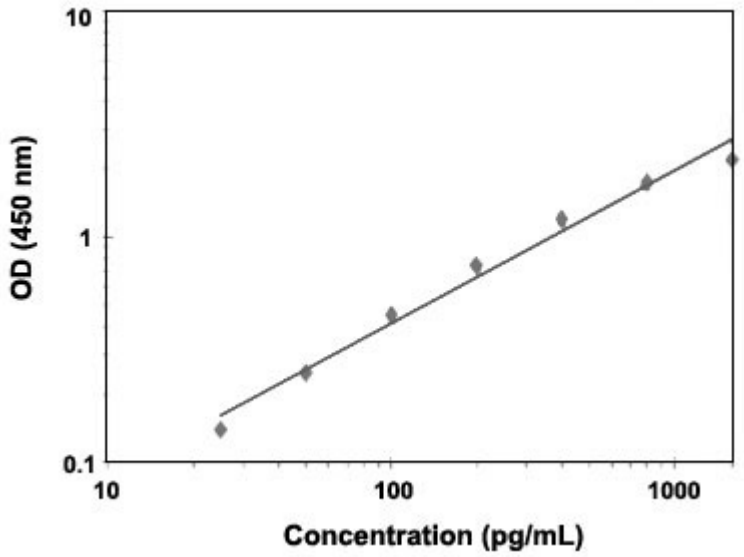

Figure 1: Typical Standard Curve for IL13RA1 (Human) ELISA Kit.

Performance Characteristics

- **Reactivity and Specificity**

The Human IL-13 Rα1 ELISA is capable of recognizing both recombinant and naturally produced Human IL-13 Rα1 proteins. The antigens listed below were tested at 50 ng/ml and did not exhibit significant cross reactivity or interference.

Human: IL-4R, IL-5Rα, IL-5Rβ, IL-9, IL-13, IL-13 Rα2

Murine: IL-13, IL-13 Rα1, IL-13 Rα2

Plate Layout

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