Human IL-13 ELISA kit

Catalog No. EZHIL13

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

Human IL-13 is an immunoregulatory cytokine produced primarily by activated Th2 lymphocytes and is a key mediator in the pathogenesis of allergic inflammation. IL-13 shares 30 % amino acid sequence homology with IL-4 and demonstrates similar biological activities. The biological activities of IL-13 include suppression of macrophage cytotoxic activity, upregulation of IL-1RA expression, and suppression of proinflammatory cytokine secretion. IL-13 has been implicated in airway hypersensitivity and mucus hypersecretion, inflammatory bowel disease, and parasitic nematode expulsion.

The Human IL-13 ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody. This kit is specifically designed for the accurate quantification of human IL-13 from cell culture supernatant, serum, plasma and other biological fluids. It is analytically validated with ready-to-use reagents.

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Kit Components

- Human IL-13 pre-Coated Plate (Part No. CS210102): One 96-well plate
- Human IL-13 Detection Antibody (Part No. CS210103): One 12 mL bottle
- Human IL-13 Standard (Part No. CS210104): One lyophilized vial
- Avidin HRP A (Part No CS210051): 12 mL bottle
- Assay Buffer A (Part No. CS210062): 25 mL bottle
- Wash Buffer (20X) (Part No. CS210053): 50 mL bottle
- Substrate Solution F (Part No. CS210054): 12 mL bottle
- Stop Solution (Part No. CS213422): 12 mL bottle
- Plate Sealers (Part No. CS210056): One 4 pack

Materials Not Supplied

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene vials

Storage and stability

Store kit and unopened components at 2℃-8℃. Do no t use kit beyond its expiration date.

Stability: Kit components are stable for a minimum of 4 months from the date of receipt if stored and handled as described below.

Reagent Precautions

- **Safety Warnings and Precautions:** This kit is designed for research use only and is not recommended for internal use in humans or animals. All chemicals should be considered potentially hazardous and principles of good laboratory practice should be followed.

- This kit is not for use in diagnostic procedures.

- Caustic Material: Stop Solution. The stop solution contains acid which is harmful if swallowed or inhaled; avoid contact with skin and eyes; wash areas of contact immediately with water. **Caution: Eye, hand, face, and clothing protection should be worn when handling this material.**

- Substrate Solution F is harmful if inhaled or ingested. Additionally, avoid skin, eye or clothing contact with the substrate reagents

- The instructions provided have been designed to optimize the kit's performance. Deviation from the instructions may result in suboptimal performance of the kit and a failure to produce accurate data.

Specimen Collection and Handling

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantification.

Cell Culture Supernatant

If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70℃. Avoid repeated freeze -thaw c ycles.

Serum

Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70℃. Avoid repeated freeze-thaw cycles.

Plasma

Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70℃.

Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

- Note: All reagents should be diluted immediately prior to use.
- Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
- Reconstitute the lyophilized Human IL-13 Standard by adding the volume of Assay Buffer A indicated on the vial label to make the 20 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
- In general, samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer A as the diluent for cell culture supernatant and Matrix A as the diluent for serum or plasma samples.

Assay Protocol

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 500 µL of the 1,000 pg/mL top standard by diluting 25 µL of the standard stock solution in 475 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 1,000 pg/mL top standard in separate tubes using As- say Buffer A as the diluent. Thus, the human IL-13 standard concentrations in the tubes are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/ mL, 31.3 pg/mL, and 15.6 pg/mL, respectively. Assay Buffer A serves as the zero standard (0 pg/mL).
4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50uL of Assay Buffer A to each well that will contain either standard dilutions or samples
6. Add 50uL of standard dilutions or samples to the appropriate wells.
7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
9. Add 100 µL of Human IL-13 Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

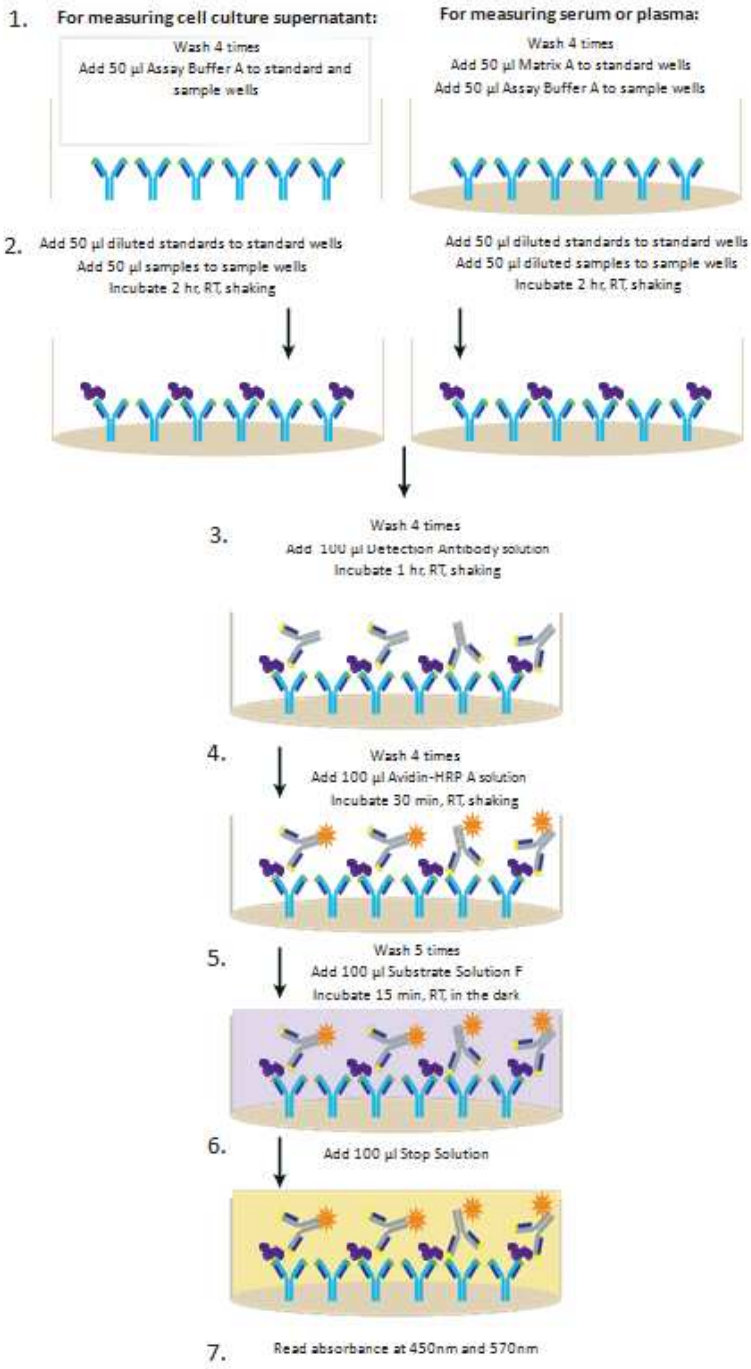
13. Add 100 µL of Substrate Solution F to each well and incubate for 15 minutes in the dark. Wells containing human IL-13 should turn blue in color with intensity proportional to its concentration. It is not necessary to seal the plate during this step.

14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

15. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

16. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards

Assay Procedure Summary



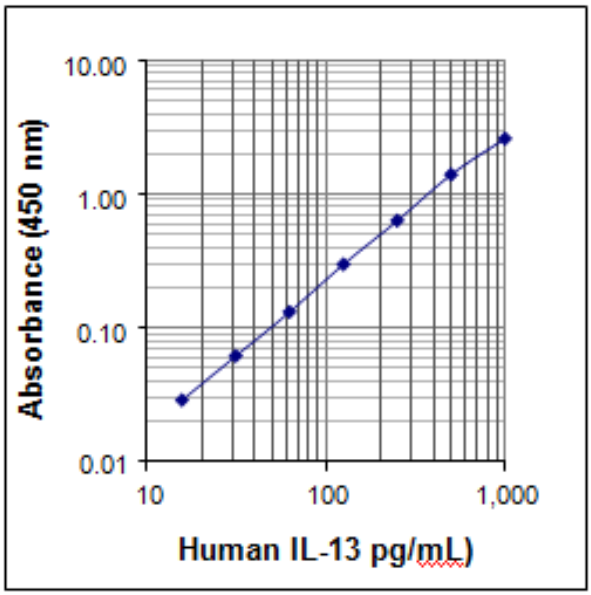
Calculations of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration 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 cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity

No cross-reactivity was observed when this kit was used to analyze mouse IL-13 and the following recombinant cytokines/chemokines at up to 50 ng/mL.
Mouse: IL-1a, IL-1b, IL-3, IL-10, IL-17A, IL-22, FGF-basic, GM-CSF, IFN-g, MCP-1/CCL2, SCF, TNF-a
Human: IL-1a, IL-1b, IL-2, IL-6, IL-8, IL-15, IL-17A, IL-22, IL-32a, FGF-basic, IFN-g, TNF-a, TSLP

Sensitivity

The minimum detectable concentration of IL-13 is 5.8 pg/mL.

Recovery

Recombinant IL-13 (1000, 250 and 62.5 pg/mL) was spiked into 10 human serum samples and analyzed with the Human IL-13 ELISA kit. On average, 96.9% of the cytokine was recovered from serum samples.

Linearity

Six human serum samples with high concentrations of IL-13 were diluted with Assay Buffer A to produce samples with values within the dynamic range of the assay. On average, 105.1% of the expected cytokine was detected from serum samples.

Intra-Assay Statistics

Sixteen replicates of each of two samples containing different IL-13 concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	490.2	142.6
Standard Deviation	18.3	11.3
% CV	3.7	7.9

Inter-Assay Statistics

Two samples containing different concentrations of IL-13 were tested in four independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	4	4
Mean Concentration (pg/mL)	504.5	139
Standard Deviation	28.7	13.6
% CV	5.7	9.8

Biological Samples

Serum/Plasma - Normal human serum and plasma (n = 33) were assayed for basal levels of human IL-13. All samples measured less than the lowest IL-13 standard curve point, 15.6 pg/mL.

Cell Culture Supernates - Human peripheral blood mononuclear cells at a concentration of $2x10^6$ cells/mL were co-stimulated with 50 ng/mL of PMA and 1 mg/mL ionomycin at 37℃ for 2 days. The cell culture supernatants were collected and assayed for the concentration of natural human IL-13. The concentration of human IL-13 was 687.9 pg/mL in PMA/ionomycin stimulated samples and undetectable in unstimulated samples.

Trouble Shooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Trouble Shooting Guide:

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

Microtiter Plate Map

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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