Human IL-13 ELISA Kit

For the quantitative determination of human interleukin 13
(IL-13) concentrations in serum, plasma,
cell culture supernatant

Catalogue Number: EL10054

96 tests

FOR LABORATORY RESEARCH USE ONLY
NOT FOR USE IN DIAGNOSTIC PROCEDURES

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S7.5 (01) IL-13

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INTENDED USE

This Human Interleukin 13 ELISA Kit is to be used for the *in vitro* quantitative determination of human interleukin 13 concentrations in cell culture supernatant. This kit is intended FOR LABORATORY RESEARCH USE ONLY and is not for use in diagnostic or therapeutic procedures.

INTRODUCTION

IL-13 is a 10kD unglycosylated protein, secreted mainly by activated T helper 2 cells. IL-13 binds to IL-4Rα in addition to IL-13Rα1 and IL-13Rα2. And the function of IL-13 partially overlaps with IL-4. Both IL-4 and IL-13 link to signal transducer and activator of transcription 6 (STAT6) through the receptor activation.

IL-13 can induce B-cell proliferation and IgE classs switching. However, IL-13 is less competent in this function comparing with IL-4. Unlike IL-4, IL-13 does not play an important role in hematopoietic cell shift, such as naïve T helper 0 differentiation to T helper 2.

IL-I3 signaling is significant in inducing physiological changes to non-immuno cells in parasitized organs. For example, IL-13 induces enhanced gut contractions and glycoprotein hypersecretion which lead to the detachment of helminthes from gut wall. Eggs produced by Schistosoma mansoni can be limited inside granulomas formed through IL-13 induced cell changes. To organisms resided inside cells, IL-13 seemed to inhibit the clearance from host cell through antagonizing Th1 responses.

IL-13 is also associated with many features of the allergic airway diseases, including mucus hypersecretion, goblet metaplasia, chemokine induction and recruitment of effector cells. All of them contribute to airway obstruction. Polymorphisms in IL-13 gene have been found to link to increased eosinophil count, serum total IgE and high risk of asthma.

PRINCIPLE OF THE ASSAY

This assay applies the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific to IL-13 has been pre-coated onto a microplate. Standards and samples are then added to the appropriate plate wells with a biotin-conjugated antibody preparation specific for IL-13 and incubated. IL-13 if present, will bind and become immobilized by the antibody pre-coated on the wells and then be "sandwiched" by biotin conjugate. Avidin is a tetramer containing four identical subunits that each has a high affinity-binding site for biotin. After washing away any unbound substances, avidin-Horseradish Peroxidase (HRP) will be added to each well and incubated. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and only those wells that contain IL-13, biotin conjugated antibody and enzyme-conjugated Avidin will exhibit a change in colour. The colour development is stopped and the intensity of the colour is measured.

In order to measure the concentration of IL-13 in the samples, this package of reagent includes two calibration diluents (Calibrator Diluent I for serum/plasma testing and Calibrator Diluent II for cell culture supernatant testing.) According to the testing system, the provided standard is diluted (2-fold) with the appropriate Calibrator Diluent and assayed at the same time as the samples. This allows the operator to produce a standard curve of Optical Density (O.D) versus IL-13 concentration. The concentration of IL-13 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

LIMITATIONS OF THE PROCEDURE

- **FOR LABORATORY RESEARCH USE ONLY, NOT FOR USE IN DIAGNOSTIC PROCEDURES**.
- The kit should not be used beyond the expiration date on the reagent label.
- It is important that the Calibration Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate Calibrator Diluent and repeat the assay.
- As manufacturers we take great care to ensure that our products are suitable for use with all validated sample types, as designated in the product insert. However, it is possible that in some cases, high levels of interfering factors may cause unusual results.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and reagent age can cause variation in binding.
- Soluble receptors or other binding proteins present in biological samples do not necessarily interfere with the measurement of ligands in samples. However, until the factors have been tested, the possibility of interference cannot be excluded.

REAGENTS PROVIDED

All reagents provided are stored at 2-8°C. Refer to the expiration date on the label.

		96 tests

1. **IL-13 Microtiter Plate** (Part EL54-1) _____ *96 wells*
    Pre-coated with anti-human IL-13 monoclonal antibody

2. **Biotin Conjugate** (Part EL54-2) _____ *6 mL*
    Anti-IL-13 polyclonal antibody conjugated to Biotin

3. **Avidin Conjugate** (Part EL54-3) _____ *12 mL*
    Avidin conjugate to horseradish peroxidase

4. **IL-13 STANDARD** (Part EL54-4) _____ *2 vials*
    Recombinant human IL-13 (2ng/vial) in a buffered protein base with
    Preservative, lyophilized.

5. **CALIBRATOR DILUENT I** (Part EL54-5) _____ *25 mL*
    Animal serum with buffer and preservative*, for serum/plasma testing.*

6. **CALIBRATOR DILUENT II** (Part EL54-6) _____ *25 mL*
    Cell culture medium with calf serum and preservative, *for cell culture supernatant testing.*

7. **WASH BUFFER (20X)** (Part 30005) _____ *60 mL*
    20-fold concentrated solution of buffered surfactant.

8. **SUBSTRATE A** (Part EL54-7) _____ *10 mL*
    Buffered solution with H_2O_2

9. **SUBSTRATE B** (Part 30007) _____ *10 mL*
    Buffered solution with TMB.

10. **STOP SOLUTION** (Part 30008) _____ *14 mL*
    2N Sulphuric Acid (H_2SO_4). Caution: Caustic Material!

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Single or multi-channel precision pipettes with disposable tips: 10-100μL and 50-200μL for running the assay.
2. Pipettes: 1 mL, 5 mL 10 mL, and 25 mL for reagent preparation.
3. Multi-channel pipette reservoir or equivalent reagent container.
4. Test tubes and racks.
5. Polypropylene tubes or containers (25 mL).
6. Erlenmeyer flasks: 100 mL, 400 mL, 1 L and 2 L.
7. Microtiter plate reader (450 nm ± 2nm).
8. Automatic microtiter plate washer or squirt bottle.
9. Sodium hypochlorite solution, 5.25% (household liquid bleach).
10. Deionized or distilled water.
11. Plastic plate cover.
12. Disposable gloves.
13. Absorbent paper.

PRECAUTIONS

1. Do not substitute reagents from one kit lot to another. Standard, conjugate and microtiter plates are matched for optimal performance. Use only the reagents supplied by manufacturer.
2. Allow kit reagents and materials to reach room temperature (20-25°C) before use. Do not use water baths to thaw samples or reagents.
3. Do not use kit components beyond their expiration date.
4. Use only deionized or distilled water to dilute reagents.
5. Do not remove microtiter plate from the storage bag until needed. Unused strips should be stored at 2-8°C in their pouch with the desiccant provided.
6. Use fresh disposable pipette tips for each transfer to avoid contamination.
7. Do not mix acid and sodium hypochlorite solutions.
8. Human serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure since no known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
9. All samples should be disposed of in a manner that will inactivate human viruses.
   Solid Wastes: Autoclave for 60 minutes at 121°C.
   Liquid Wastes: Add sodium hypochlorite to a final concentration of 1.0%. The waste should be allowed to stand for a minimum of 30 minutes to inactivate viruses before disposal.
10. Substrate Solution is easily contaminated. If bluish prior to use, *do not use*.
11. Substrate B contains 20% acetone: Keep this reagent away from sources of heat and flame.
12. If Wash Buffer (20X) is stored at a lower temperature (2-5°C), crystals may form which must be dissolved by warming to 37°C prior to use.

SAMPLE PREPARATION

COLLECTION, HANDLING AND STORAGE

a) **Cell Culture Supernatant:** Centrifuge to remove any visible particulate material.
b) **Serum**: Blood should be drawn using standard venipuncture techniques and serum separated from the blood cells as soon as possible. Samples should be allowed to clot for one hour at room temperature, centrifuged for 10 minutes (4°C), and serum extracted.
a) **Plasma:** Blood should be drawn using standard venipuncture techniques and plasma collected using sodium citrate, EDTA, or heparin as an anticoagulant. To ensure optimal recovery and minimal platelet contamination, separation of plasma must be done on ice in less than 30 minutes after collection. Centrifuge for 10 minutes (4°C) to remove any particulate.

- Avoid grossly hemolytic, lipidic or turbid samples.
- Serum, plasma, and cell culture supernatant to be used within 24-48 hours may be stored at 2-8°C, otherwise samples must stored at -20°C to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles.
- When performing the assay, slowly bring samples to room temperature.
- It is recommended that all samples be assayed in duplicate.
- DO NOT USE HEAT-TREATED SPECIMENS.
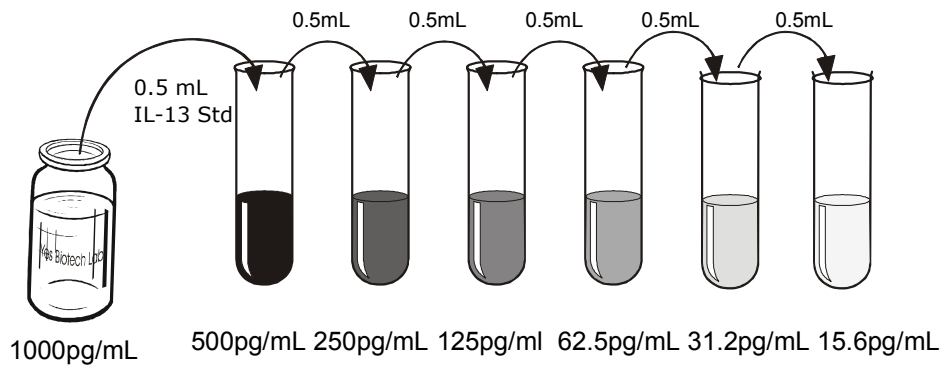

PREPARATION OF REAGENTS

Remove all kit reagents from refrigerator and allow them to reach room temperature (20-25°C). Prepare the following reagents as indicated below. Mix thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Wash Buffer (1X):** Add 60 mL of Wash Buffer (20X) and dilute to a final volume of 1200 mL with distilled or deionized water. Mix thoroughly. If a smaller volume of Wash Buffer (1X) is desired, add 1 volume of Wash Buffer (20X) to 19 volumes of distilled or deionized water. Wash Buffer (1X) is stable for 1 month at 2-8°C. Mix well before use.

2. **Substrate Solution:** Substrate A and Substrate B should be mixed together in equal volumes up to 15 minutes before use. Refer to the table provided for correct amounts of Substrate Solution to prepare.

Strips Used	Substrate A (mL)	Substrate B (mL)	Substrate Solution (mL)
2 strips (16 wells)	2.0	2.0	4.0
4 strips (32 wells)	3.0	3.0	6.0
6 strips (48 wells)	4.0	4.0	8.0
8 strips (64 wells)	5.0	5.0	10.0
10 strips (80 wells)	6.0	6.0	12.0
12 strips (96 wells)	7.0	7.0	14.0

3. **IL-13 Standard:**

a) Two vials of Standard are provided in this package to allow both serum/plasma and cell culture supernatant testing. Reconstitute IL-13 Standard with 2.0 mL of Calibrator Diluent I or Calibrator Diluent II. This reconstitution produces a stock solution of 1ng/mL. Allow solution to sit for at least 15 minutes with gentle agitation prior to making dilutions. Use within one hour of reconstituting. The IL-13 standard stock solution can be stored frozen (-20°C) for up to 10 days. Avoid freeze-thaw cycles: aliquot if repeated use is expected.

b) Use the above stock solution to produce a serial 2-fold dilution series, as described below, within the range of this assay (1000pg/mL to 15.6pg/mL) as illustrated. Add 0.5 mL of the appropriate Calibrator Diluent to each test tube. Between each test tube transfer, be sure to mix contents thoroughly. The undiluted IL-13 stock solution will serve as the high standard (1000pg/mL) and the Calibrator Diluent will serve as the zero standard (0ng/mL).

ASSAY PROCEDURE

1. Prepare Wash Buffer (1X) and IL-13 Standards before starting assay procedure (see Preparation of Reagents). *It is recommended that the table and diagram provided be used as a reference for adding Standards or Samples to the Microtiter Plate.*

Wells	Contents	Wells	Contents
1A, 1B	Standard 1 - **0 pg/mL** (S1)	**2C, 2D**	Standard 6 - **250pg/mL** (S6)
1C, 1D	Standard 2 – **15.6pg/mL** (S2)	**2E, 2F**	Standard 7 - **500pg/mL** (S7)
1E, 1F	Standard 3 – **31.2pg/mL** (S3)	**2G, 2H**	Standard 8 - **1000pg/mL** (S8)
1G, 1H	Standard 4 – **62.5pg/mL** (S4)	**3A-12H**	**IL-13** samples
2A, 2B	Standard 5 – **125pg/mL** (S5)		

	1	2	3	4	5	6	7	8	9	10	11	12
A	S1	S5	1	5	9	13	17	21	25	29	32	36
B	S1	S5	1	5	9	13	17	21	25	29	32	37
C	S2	S6	2	6	10	14	18	22	26	30	33	37
D	S2	S6	2	6	10	14	18	22	26	30	33	38
E	S3	S7	3	7	11	15	19	23	27	31	34	39
F	S3	S7	3	7	11	15	19	23	27	31	34	39
G	S4	S8	4	8	12	16	20	24	28	32	35	40
H	S4	S8	4	8	12	16	20	24	28	32	35	40

2. Add 100μL of Standard or Sample to the appropriate well of the antibody pre-coated Microtiter Plate and incubate <u>1 hour at room temperature.</u>

3. Without discarding the standards and samples, add 50μL IL-13 Biotin conjugate to each wells. Mix well. Cover and incubate for 1 <u>hour at room temperature.</u>

4. Wash the Microtiter Plate using one of the specified methods indicated below:

   <u>Manual Washing</u>: Remove incubation mixture by aspirating contents of the plate into a sink or proper waste container. Using a squirt bottle, fill each well completely with Wash Buffer (1X) then aspirate contents of the plate into a sink or proper waste container. Repeat this procedure four more times for a **total of FIVE washes**. After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *Note*: Hold the sides of the plate frame firmly when washing the plate to assure that all strips remain securely in frame.
   <u>Automated Washing</u>: Aspirate all wells, then wash plates **FIVE times** using Wash Buffer (1X). Always adjust your washer to aspirate as much liquid as possible and set fill volume at 350 μL/well/wash (range: 350-400 μL). After final wash, invert plate, and blot dry by hitting plate onto absorbent paper or paper towels until no moisture appears. *It is recommended that the washer be set for a soaking time of 10 seconds or shaking time of 5 seconds between washes.*

5. Dispense 100μl of Avidin Conjugate to each well Mix well. Cover and incubate for <u>1 hour at room temperature</u>.

6. Prepare Substrate Solution no more than 15 minutes before end of second incubation (see Preparation of Reagents).

7. Repeat wash procedure as described in Step 4.

8. Add 100μL Substrate Solution to each well. Cover and incubate for <u>15 minutes at room temperature.</u>

9. Add 100μL Stop Solution to each well. Mix well.

10. Read the Optical Density (O.D.) at 450 nm using a microtiter plate reader set within 30 minutes.

CALCULATION OF RESULTS

The standard curve is used to determine the amount of IL-13 in an unknown sample. The standard curve is generated by plotting the average O.D. (450 nm) obtained for each of the standard concentrations on the vertical (Y) axis versus the corresponding IL-13 concentration (ng/mL) on the horizontal (X) axis.

1. First, calculate the mean O.D value for each standard and sample. All O.D. values are subtracted by the value of the zero-standard (0 ng/mL) or (S1) before result interpretation. Construct the standard curve using graph paper or statistical software.

2. To determine the amount of IL-13 in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding IL-13 Rα2 concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

3. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
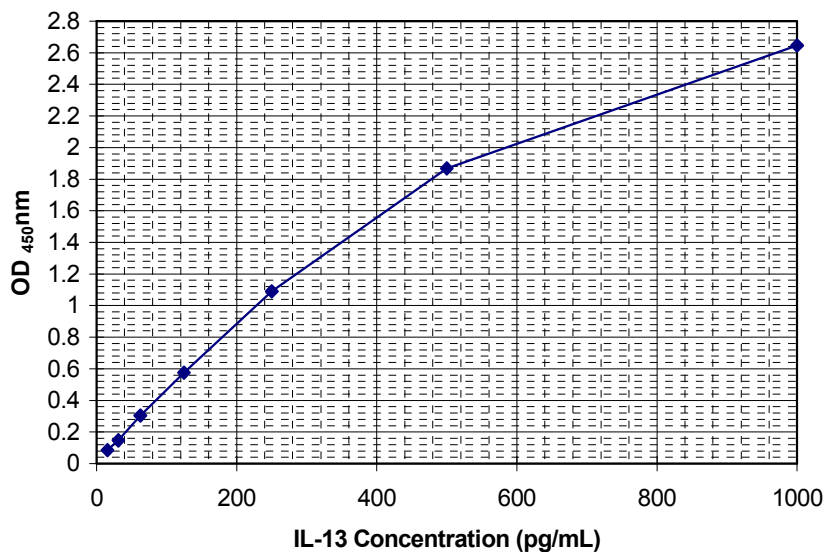
TYPICAL DATA

Results of a typical standard run of an IL-13 ELISA are shown below. Any variation in standard diluent, operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. The following examples are for the purpose of _illustration only_, and should not be used to calculate unknowns. Each user should obtain its own result.

<u>EXAMPLE</u>
The following data was obtained for a standard curve using Calibrator Diluent I.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.) - (S1)
0	0.0595	3.570	0
15.6	0.1445	3.430	0.0850
31.25	0.2065	2.400	0.1470
62.5	0.3635	1.360	0.3040
125	0.6350	0.068	0.5755
250	1.1500	3.200	1.0905
500	1.9270	0.081	1.8675
1000	2.7055	0.026	2.6460

The following data was obtained for a standard curve using Calibrator Diluent II.

Standard (pg/mL)	Mean O.D. (450 nm)	%CV	Zero Standard Subtracted (Std.) - (S1)
0	0.0615	5.75	0.0000
15.6	0.1270	3.34	0.0655
31.25	0.1975	3.94	0.1360
62.5	0.3175	0.67	0.2560
125	0.5715	2.85	0.5100
250	1.0075	0.07	0.9460
500	1.6960	2.76	1.6345
1000	2.4060	1.59	2.3445
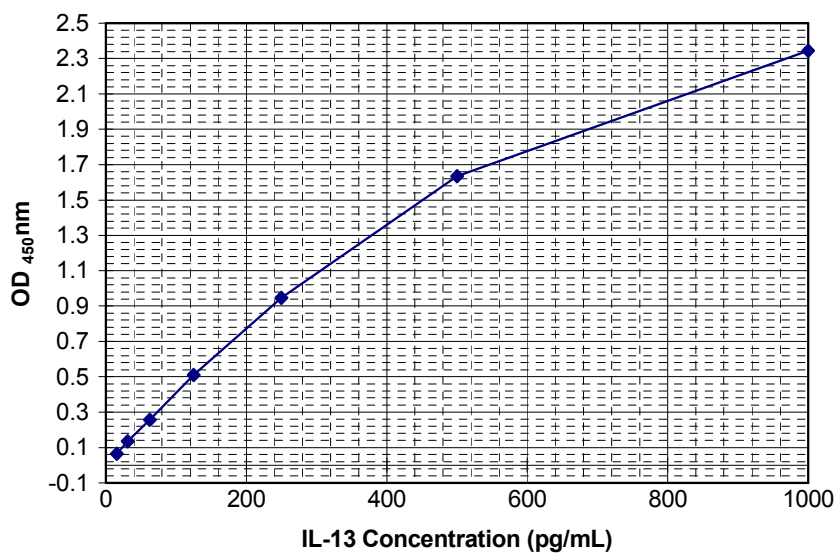
PRECISION

1. INTER-ASSAY PRECISION

To determine within-run precision, three different samples of known concentration were assayed by replicates of 8 in 1 assay.

Sample	1	2	3
n	**8**	**8**	**8**
Mean (pg/mL)	134.3	276.4	563.7
Standard Deviation (pg/mL)	0.013	0.033	0.16
Coefficient of Variation (%)	*2.14*	*2.91*	*8.04*

2. **INTER-ASSAY PRECISION**

To determine between-run precision, three different samples of known concentration were assayed by replicates on 8 different assays.

Sample	1	2	*3*
n	**8**	**8**	**8**
Mean (pg/mL)	142.3	280	567.6
Standard Deviation (pg/mL)	0.07	0.19	0.18
Coefficient of Variation (%)	*3.25*	*7.05*	*5.05*

SENSITIVITY

The sensitivity of human IL-13 as observed by the standard curve generated from Calibratory Diluent 1 is about 1pg/ml. It is defined as the detected quantity 2SD from the mean OD of 20 replicates of the zero standard.

SPECIFICITY

This sandwich ELISA recognises both natural and recombinant human IL-13 Ra2. This kit has been tested and exhibited no significant cross-reactivity with following cytokines and growth factors: IL-1 α, IL-1β, IL-2,   IL-2sRa, IL-4,   IL-5, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, Il-17A, IL-15, FGF basic, GM-CSF, IFN-γ, M-CSF, MCP-1(MCAF), MCP3, EGF, TNF- α, TNF- β.

EXPECTED VALUE IN NORMAL HUMAN BLOOD SAMPLES

106 normal human serum and plasma samples were tested for IL-13 with this kit. The values were lower than 12pg/ml.

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