Human IL-13 ELISA Kit

Catalog Number
EK0447

For the quantitative determination of human Interleukin 13 (IL-13) concentrations in cell culture supernates, serum, and plasma.

This instruction must be read in its entirety before using this product.

For research use only. Not for use in diagnostic procedures.

Contact information

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PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for IL-13 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-13 present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for IL-13 is added to the wells and binds to the combination of capture antibody- IL-13 in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps a substrate is added. A coloured product is formed in proportion to the amount of IL-13 present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450nm. A standard curve is prepared from seven IL-13 standard dilutions and IL-13 sample concentration determined.

DETECTION RANGE

3.125 pg/ml - 200 pg/ml

SENSITIVITY

The minimum detectable dose was 1pg/mL.

SPECIFICITY

This assay recognizes both natural and recombinant human IL-13. The factors listed below were prepared at 50ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Table 1: Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant rat
IL-4	IL-4	IL-4
IL-4I1	IL-4I1	IL-13
IL-4 R α	IL-4 R α	
IL-13 R α 1	IL-13	

PRECISION

The coefficient of variation of both intra-assay and inter-assay were less than 10%.

MATERIALS PROVIDED

1. Aluminium pouches with a Microwell Plate coated with antibody to human IL-13 (8X12)
2. 2 vials human IL-13 Standard lyophilized, 200 pg/ml upon reconstitution
3. 2 vials concentrated Biotin-Conjugate anti-human IL-13 antibody
4. 2 vials Streptavidin-HRP solution
5. 1 bottle Standard /sample Diluent
6. 1 bottle Biotin-Conjugate antibody Diluent
7. 1 bottle Wash Buffer Concentrate 20x (PBS with 1% Tween-20)
8. 1 vial Substrate Solution
9. 1 vial Stop Solution
10. 4 pieces Adhesive Films
11. package insert

STORAGE

Table 2: Storage of the kit

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2 – 8℃. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="9">Opened/ Reconstituted Reagents</td><td>Standard /sample Diluent</td><td rowspan="7">May be stored for up to 1 month at 2 – 8℃.**</td></tr>
<tr><td>Concentrated Biotin-Conjugate</td></tr>
<tr><td>Streptavidin-HRP solution</td></tr>
<tr><td>Biotin-Conjugate antibody Diluent</td></tr>
<tr><td></td></tr>
<tr><td>Wash Buffer Concentrate 20x</td></tr>
<tr><td>Substrate Solution</td></tr>
<tr><td>Stop Solution</td><td rowspan="2">Aliquot and store for up to 1 month at -20℃.
Avoid repeated freeze-thaw cycles. Diluted standard shall not be reused.</td></tr>
<tr><td>Standard</td></tr>
<tr><td></td><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the</td></tr>
</table>

		desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 – 8℃.**

**Provided this is within the expiration date of the kit

THE REQUIRED ITEMS (not provided, but can help to buy):

1. Microplate reader (450nm).
2. Micro-pipette and tips: 0.5-10, 2-20, 20-200, 200-1000μL.
3. 37 ℃ incubator, double-distilled water or deionized water, coordinate paper, graduated cylinder.

PRECAUTIONS FOR USE

1. Store kit regents between 2℃and 8℃. After use all reagents should be immediately returned to cold storage(2℃ to 8℃).
2. Please perform simple centrifugation to collect the liquid before use.
3. To avoid cross contamination, please use disposable pipette tips.
4. The Stop Solution suggested for use with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material. Avoid contact of skin or mucous membranes with kit reagents or specimens. In the case of contact with skin or eyes wash immediately with water.
5. Use clean, dedicated reagent trays for dispensing the washing liquid, conjugate and substrate reagent. Mix all reagents and samples well before use.
6. After washing microtiter plate should be fully pat dried. Do not use absorbent paper directly into the enzyme reaction wells.
7. Do not mix or substitute reagents with those from other lots or other sources. Do not use kit reagents beyond expiration date on label.
8. Each sample, standard, blank and optional control samples should be assayed in duplicate or triplicate.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency or Shake by hand at 10min interval when there is no vortexer.
10. Avoid microtiter plates drying during the operation.

11. Dilute samples at the appropriate multiple, and make the sample values fall within the standard curve. If samples generate values higher than the highest standard, dilute the samples and repeat the assay.
12. Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time and temperature, and kit age can cause variation in binding.
13. This method can effectively eliminate the interference of the soluble receptors, binding proteins and other factors in biological samples.

SAMPLE COLLECTION AND STORAGE

1. **Cell Culture Supernates** - Remove particulates by centrifugation.
2. **Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 x g. Remove serum, avoid hemolysis and high blood lipid samples.
3. **Plasma** - Recommended EDTA as an anticoagulant in plasma. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection.
4. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
5. Dilute samples at the appropriate multiple (recommended to do pre-test to determine the dilution factor).
   Note: The normal human serum or plasma samples are suggested to make a 1:2 dilution.

REAGENT PREPARATION

1. Bring all reagents to room temperature before use.
2. **Wash Buffer** - Dilute 10mL of Wash Buffer Concentrate into deionized or distilled water to prepare 200mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mix gently until the crystals have completely dissolved.
3. **Standard** - Reconstitute the Standard with 0.5mL of Standard /sample Diluent. This reconstitution produces a stock solution of 1000 pg /mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

   Pipette 250µL of Standard/sample Diluent into the 100 pg/mL tube and

the remaining tubes. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 200 pg/mL standard serves as the high standard. The Standard/ sample Diluent serves as the zero standard (0 pg/mL).

If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.

4. Working solution of **Conjugate Mixture**: Dilute Biotin-Conjugate(1:100) and Streptavidin-HRP(1:250) with Biotin-Conjugate antibody Diluent before use.

The working solution should be used within one day after dilution.

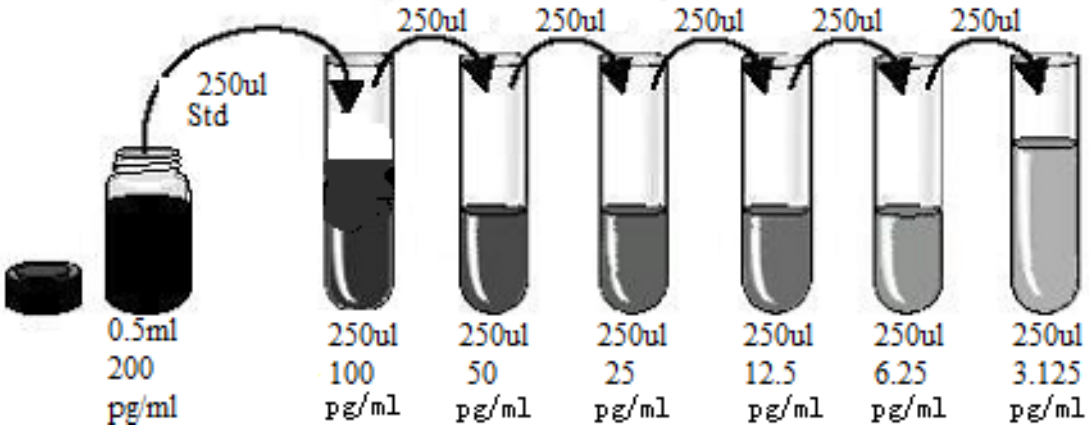

Figure 1: Preparation of IL-13 standard dilutions

GENERAL ELISA PROTOCOL

1. Prepare all reagents and working standards as directed in the previous sections.
2. Determine the number of microwell strips required to test the desired number of samples plus appropriate number of wells needed for running blanks and standards. Remove extra microwell strips from holder and store in foil bag with the desiccant provided at 2-8℃ sealed tightly.
3. Add 100µL of Standard, control, or sample ,per well,then add 50µL of prepared Conjugate Mixture to all wells. Cover with the adhesive strip provided. Incubate for 2 hours at RT. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
4. Aspirate each well and wash, repeating the process three times for a

total of four washes. Wash by filling each well with Wash Buffer (350μL) using a squirt bottle, manifold dispenser or auto-washer. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

5. Add 100μL of Substrate Solution to each well. Incubate for 20-30 minutes at RT. Avoid placing the plate in direct light.
6. Add 100μL 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 450 nm.(optionally 650nm as the reference wave length;610-650nm is acceptable)

ASSAY PROCEDURE SUMMARY

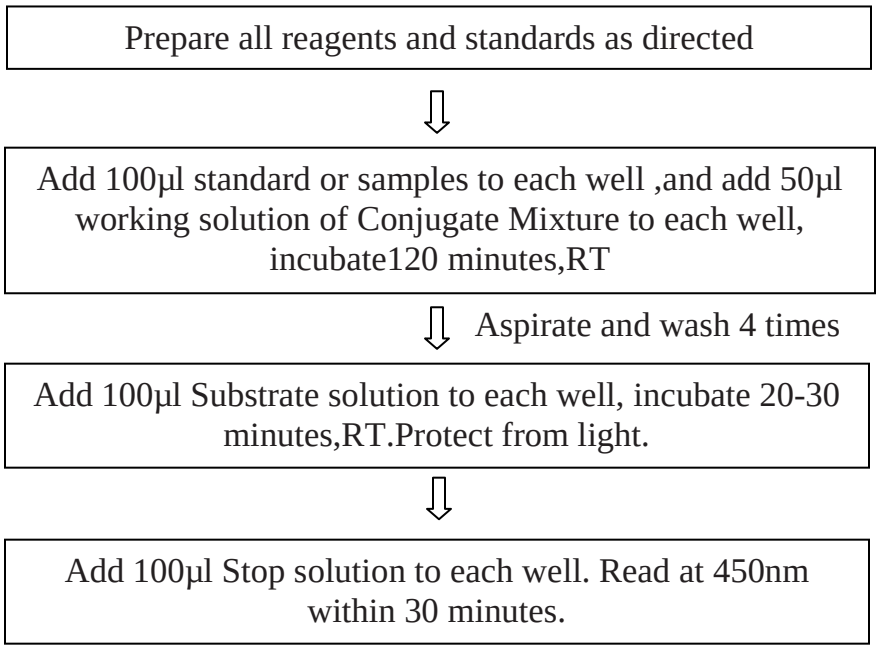

Figure 2: Assay procedure summary

TECHNICAL HINTS

1. When mixing or reconstituting protein solutions, always avoid foaming.

2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
4. Substrate Solution should remain colorless until added to the plate. Stop Solution should be added to the plate in the same order as the Substrate Solution. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
5. A standard curve should be generated for each set of samples assayed. According to the content of tested factors in the sample, appropriate diluted or concentrated samples, it is best to do pre-experiment.

CALCULATION OF RESULTS

1. Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.
2. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, 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.
3. The data may be linearized by plotting the log of the IL-13 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
4. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Table 3:Typical data using the IL-13 ELISA (Measuring wavelength:450nm,Reference wavelength:650nm)

Standard (pg/ml)	OD.	OD.	Average	Corrected
0	0.167	0.164	0.166	——
3.125	0.193	0.187	0.190	0.024
6.25	0.205	0.219	0.212	0.046
12.5	0.316	0.302	0.309	0.143
25	0.444	0.432	0.438	0.272
50	0.613	0.599	0.606	0.440
100	0.970	0.964	0.967	0.801
200	1.827	1.819	1.823	1.657

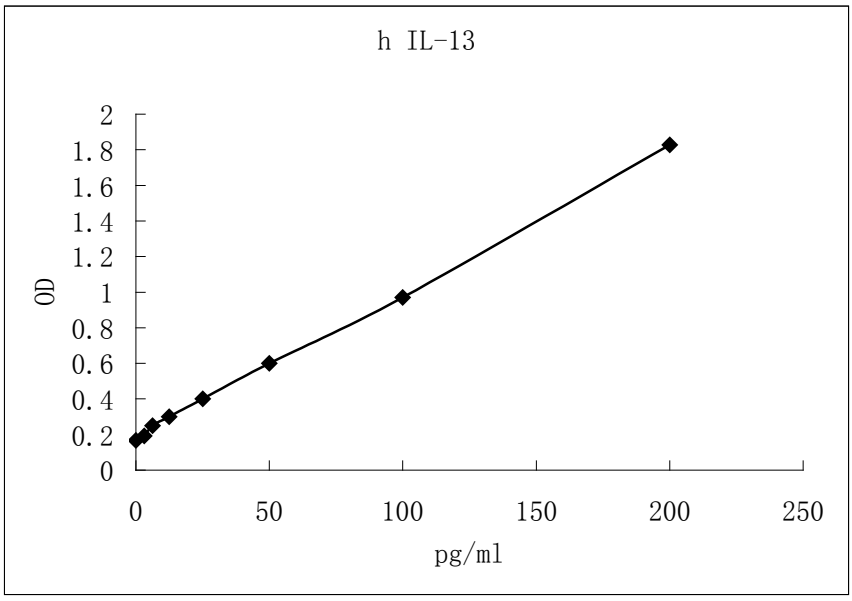

Figure 3:Representative standard curve for IL-13 ELISA. IL-13 was diluted in serial two-fold steps in Sample Diluent.

Do not use this standard curve to derive test results. A standard curve must be run for each group of microwell strips assayed.

BACKGROUND

Interleukin-13 (IL-13) is a 17 kDa immunoregulatory cytokine that plays a key role in the pathogenesis of allergy, cancer, and tissue fibrosis (1-3). It is secreted by Th1, Th2, Th17, NK, and mast cells, visceral smooth muscle cells, eosinophils, and basophils (2, 4). This pattern is similar to the expression of Interleukin-4 (IL-4) but also includes subsets of Th1 and Th17

cells that do not secrete IL-4 (5). IL-13 circulates as a monomer and has two internal disulfide bonds that contribute to its bundled four α-helix configuration (6, 7). Mature human IL-13 shares approximately 58% amino acid sequence identity with mouse and rat IL-13. Despite the low homology, it exhibits cross-species activity between human, mouse, and rat (8, 9). IL-13 suppresses the production of proinflammatory cytokines and other cytotoxic substances by macrophages, fibroblasts, and endothelial cells. On B cells, it promotes cellular activation, immunoglobulin class switching to IgE, and the upregulation of CD23/FcεRII. Polymorphisms and upregulation of IL-13 are associated with atopy, asthma, airway hyperresponsiveness, and tissue fibrosis (1, 10). The biological effects of IL-13 and IL-4 are closely related due in part to a shared receptor system. IL-13 binds with low affinity to the transmembrane IL-13 Rα1 which then forms a signaling complex with the transmembrane IL-4 Rα (11-13). This high affinity receptor complex also functions as the type 2 IL-4 receptor (11, 12). Soluble forms of IL-4 Rα are expressed which retain ligand binding properties and inhibit IL-4 bioactivity (14, 15). IL-4 Rα also associates with the common gamma chain (γc) to form the type 1 IL-4 receptor complex (16, 17). Additionally, IL-13 binds with high affinity to IL-13 Rα2 which is expressed as cell surface and soluble forms (18-20). IL-13 Rα2 functions as a decoy receptor by preventing IL-13 from signaling through the IL-13 Rα1/IL-4 Rα complex (21, 22). It also inhibits responsiveness to IL-4 by blocking signaling through IL-4-occupied IL-13 Rα1/IL-4 Rα receptor complexes (22, 23). IL-13 Rα2 is upregulated during Th2-biased immune responses and limits inflammatory tissue damage (20, 24, 25). Aside from its decoy function, IL-13 Rα2 can signal in response to IL-13 to directly promote tumor cell invasiveness and the development of tissue fibrosis (26-28).

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