ELISA Kit
Catalog #KMC2221

Mouse
IL-13

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PURPOSE

The Invitrogen Mouse Interleukin-13 (Ms IL-13) ELISA is to be used for the quantitative determination of IL-13 in mouse serum and cell culture medium. The assay will recognize both natural and recombinant Ms IL-13.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Read entire protocol before use.

INTRODUCTION

Interleukin-13 (IL-13), a cytokine produced primarily by Th2 cells, is implicated in asthma, allergy, and host responses to gastrointestinal parasites. IL-13 was identified by differential screening of a subtracted cDNA library prepared from mononuclear cells stimulated with antibody directed to CD28. Mouse IL-13, known alternatively as P600, is comprised of 111 amino acid residues with variable molecular mass due to differential glycosylation. The accession numbers for the mouse IL-13 protein and gene are P20109 and L13028, respectively. The mouse IL-13 protein is 58% homologous with human IL-13 and 63% homologous with rat IL-13.

At least two receptors for IL-13 have been identified: IL-13Rα1 and IL-4Rα. Both IL-13Rα1 and IL-4Rα interact with the common gamma chain of the IL-2 receptor, although the IL-13Rα1/IL-4Rα complex appears to be sufficient for serving as a high affinity IL-13 receptor. While the production of IL-13 appears to be limited to cells of the

immune system, IL-13Rα1 is found to be widely expressed by many cell types. IL-13 binding to its receptors activates members of the JAK family, which in turn phosphorylate and activate STAT6, resulting in enhanced transcription of responsive genes. IL-13 is also observed to induce the phosphorylation of IRS1/2, to activate PI-3 kinase, to mobilize intracellular Ca^{2+} stores, and to elevate cAMP.

IL-13 also binds to a putative decoy receptor, designated IL-13 Binding Protein (IL-13 BP) or IL-13Rα2, found on the cell surface, and in serum and urine of mice. IL-13 BP is structurally distinct from IL-13Rα1, and binds to IL-13 with 100-300 fold higher affinity. By sequestering circulating IL-13, IL-13 BP modulates IL-13's biological activity by inhibiting its binding to signal transducing, cell surface-associated receptors.

IL-13 is observed to exert several effects on responding cells. With macrophages and monocytes, IL-13 suppresses nitric oxide production and reduces the production of pro-inflammatory cytokines including IL-1, IL-6, IL-8, IL-10, IL-12 and the chemokines MIP and MCP in response to IFN-g or LPS stimulation. With B cells, IL-13 enhances proliferation and immunoglobulin class switching to IgE, MHC class II expression, and CD23 expression. IL-13 also influences Th2 cell development and may be important in Th2 cell commitment.

PRINCIPLE OF THE METHOD

The Invitrogen Mouse IL-13 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A monoclonal antibody specific for Ms IL-13 has been coated onto the wells of the microtiter strips provided. Samples, including standards of known Ms IL-13 content, control specimens, and unknowns, are pipetted into these wells.

During the first incubation, the Ms IL-13 antigen binds to the immobilized (capture) antibody on one site. After washing, a biotinylated antibody specific for Ms IL-13 is added. During the second incubation, this antibody binds to the immobilized Ms IL-13 captured during the first incubation.

After removal of excess second antibody, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a third incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Ms IL-13 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	*96 Test Kit*
Ms IL-13 Standard, recombinant Ms IL-13, lyophilized. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 8 mM sodium azide; 50 mL per bottle.	1 bottle
Ms IL-13 High and Low Control, recombinant Ms IL-13, lyophilized. Refer to vial label for reconstitution volume and range.	2 vials
Ms IL-13 Antibody-Coated Wells, 96 wells per plate.	1 plate
Ms IL-13 Biotin Conjugate (Biotin-labeled anti-Ms IL-13). Contains 8 mM sodium azide; 11 mL per bottle.	1 bottle
Extraction Solution; 10 mL per bottle.	1 bottle
Streptavidin-Peroxidase (HRP), (100x) concentrate. Contains 0.05% Proclin® 300; 0.125 mL per vial.	1 vial
Streptavidin-Peroxidase (HRP) Diluent. Contains 0.04% Proclin® 300; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25x); 100 mL per bottle.	1 bottle
Stabilized Chromogen, Tetramethylbenzidine (TMB); 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	4

Disposal Note: This kit contains materials with small quantities of sodium azide. Sodium azide reacts with lead and copper plumbing to form explosive metal azides. Upon disposal, flush drains with a large volume of water to prevent azide accumulation. Avoid ingestion and contact with eyes, skin and mucous membranes. In case of contact, rinse affected area with plenty of water. Observe all federal, state and local regulations for disposal.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.
9. Polypropylene tubes for sample extraction.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic sera. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Samples that are >250 pg/mL should be diluted with *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 2 hours of assay completion.
13. The provided controls should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with *Wash Buffer* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

REAGENT PREPARATION AND STORAGE

The Ms IL-13 standard was calibrated against a highly purified recombinant protein expressed in *E. coli* at Invitrogen.

A. Reconstitution and Dilution of Ms IL-13 Standard

Note: Polypropylene tubes may be used for standard dilutions.

1. Reconstitute standard to 2,500 pg/mL with *Standard Diluent Buffer*. Refer to standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. It is recommended that standard be used within 1 hour of reconstitution.
2. Add 0.1 mL of the reconstituted standard to a tube containing 0.900 mL *Standard Diluent Buffer*. Label as 250 pg/mL Ms IL-13. Mix.
3. Add 0.500 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 125, 62.5, 31.3, 15.6, 7.8 and 3.9 pg/mL Ms IL-13.
4. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Mouse IL-13 Standard

Standard:	Add:	Into:
250 pg/mL	Prepare as described in Step 2.	
125 pg/mL	0.500 mL of the 250 pg/mL std.	0.500 mL of the Diluent Buffer
62.5 pg/mL	0.500 mL of the 125 pg/mL std.	0.500 mL of the Diluent Buffer
31.3 pg/mL	0.500 mL of the 62.5 pg/mL std.	0.500 mL of the Diluent Buffer
15.6 pg/mL	0.500 mL of the 31.3 pg/mL std.	0.500 mL of the Diluent Buffer
7.8 pg/mL	0.500 mL of the 15.6 pg/mL std.	0.500 mL of the Diluent Buffer
3.9 pg/mL	0.500 mL of the 7.8 pg/mL std.	0.500 mL of the Diluent Buffer
0 pg/mL	0.500 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Reconstitution of Ms IL-13 Controls

Reconstitute controls with 1 mL distilled water. Refer to controls vial labels for values and acceptable ranges in pg/mL. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Once reconstituted, aliquot and store at –20°C or below. Avoid repeated freeze-thaw cycles. Do not extract controls.

D. Storage and Final Dilution of Streptavidin-HRP

1. Dilute 10 µL of this 100x concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.
2. Return the unused *Streptavidin-HRP* concentrate to the refrigerator.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP Concentrate	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

E. Dilution of Wash Buffer

Allow the 25x concentrate to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the 25x wash buffer concentrate with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

SAMPLE EXTRACTION FOR SERUM AND CELL CULTURE
This step allows for the release of Ms IL-13 from IL-13 Binding Protein, making it accessible for measurement in the immunoassay.

In a polypropylene tube, add:
50 µL of each sample.
50 µL of Extraction Solution.

Vortex. Incubate 10 minutes at room temperature. Add 400 µL of the *Standard Diluent Buffer* (dilution factor: 10 fold).

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 200 µL of the *Standard Diluent Buffer* to zero wells. Well(s) reserved for chromogen blank should be left empty.
3. For the standard curve, add 200 µL of standard to the appropriate microtiter wells. For samples (after extraction) or controls add 200 µL of sample to each well. Tap gently on the side of plate to thoroughly mix. (See **REAGENT PREPARATION AND STORAGE**, Section B).
4. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.
5. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
6. Pipette 100 µL of biotinylated anti-Ms IL-13 (*Biotin Conjugate*) solution into each well except the chromogen blank(s). Tap gently on the side of the plate to mix.
7. Cover plate with *plate cover* and incubate for **1 hour at room temperature.**
8. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
9. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE,** Section C.
10. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.

11. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
12. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
13. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.
14. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
15. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution.* Read the plate within 2 hours after adding the *Stop Solution.*
16. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth

curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.

17. Read the Ms IL-13 concentrations for unknown samples and controls from the standard curve plotted in step 16. **Multiply value(s) obtained for sample(s) by 10 to correct the 1:10 extraction dilution factor.** (Samples producing signals greater than that of the highest standard (250 pg/mL) should be diluted in *Standard Diluent Buffer* for serum/plasma samples or corresponding medium for cell culture samples and reanalyzed, multiplying the concentration found by the appropriate dilution factor.

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 250 pg/mL Ms IL-13.

Standard Ms IL-13 (pg/mL)	Optical Density (450 nm)
0	0.076
	0.072
3.9	0.151
	0.147
7.8	0.233
	0.224
15.6	0.386
	0.386
31.3	0.633
	0.582
62.5	1.192
	1.198
125	1.854
	1.883
250	3.088
	3.120

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 250 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples >250 pg/mL with *Standard Diluent Buffer; reanalyze these and multiply results by the appropriate dilution factor*.

The influence of various drugs, aberrant sera (hemolyzed, hyperlipidemic, jaundiced, etc.) and the use of biological fluids in place of serum samples have not been thoroughly investigated. The rate of degradation of native Ms IL-13 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Ms IL-13 is <2 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

PRECISION

1. Intra-Assay Precision

Samples of known Ms IL-13 concentrations were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	12.37	46.20	150.50
SD	0.98	2.49	10.10
%CV	8.0	5.0	7.0

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 40 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	12.98	47.67	154.90
SD	1.44	3.09	11.50
%CV	11.0	6.0	7.0

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Mouse serum and cell culture samples were serially diluted in *Standard Diluent Buffer* or *RPMI* containing 1% fetal calf *serum*, respectively, over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded an average correlation coefficient of 0.999.

RECOVERY

The recovery of Ms IL-13 added to mouse serum averaged 96%. The recovery of Ms IL-13 added to tissue culture medium containing 1% fetal calf serum averaged 109%, while the recovery of Ms IL-13 added to tissue culture medium containing 10% fetal calf serum averaged 106%.

SPECIFICITY

Buffered solutions of a panel of substances at 50 ng/mL were assayed with the Invtrogen Ms IL-13 kit. The following substances were tested and found to have no cross-reactivity: mouse IFN-γ, IL-4, IL-12, IL-15, IL-18, VEGF, EGF, EOTAXIN, RANTES, MIP-1β, MCP-1, MIP-2; human IL-13; rat IL-13.

HIGH DOSE HOOK EFFECT

A sample spiked with 1.0 μg/mL of Ms IL-13 gave a response higher than that obtained for the last standard point.

EXPECTED VALUES

A limited number (n=11) of mouse sera were assayed with the Invitrogen Mouse IL-13 kit. The mean value obtained was 50 pg/mL (range: 15 to 93 pg/mL).

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

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Mouse IL-13 Assay Summary