Mouse IL-13 ELISA Kit

Catalog Number KMC2221 (96 tests)

Pub. No. MAN0014366 **Rev.** 2.0

CAUTION! 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.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The Invitrogen™ Mouse IL-13 ELISA Kit is a solid-phase sandwich Enzyme-Linked Immunosorbent Assay (ELISA) designed to detect and quantify the level of mouse IL-13 in mouse serum and cell culture medium. The assay will recognize both natural and recombinant mouse IL-13.

Mouse IL-13 protein is 58% homologous with human IL-13 and 63% homologous with rat IL-13.

Contents and storage

Upon receipt, store the kit at 2°C to 8°C.

Contents	Cat. No. KMC2221 (96 tests)
Ms IL-13 Standard, lyophilized. Refer to vial label for quantity and reconstitution volume	2 vials
Standard Diluent Buffer; contains 8 mM sodium azide	50 mL
Ms IL-13 High and Low Control; lyophilized. Refer to vial label for reconstitution volume and range	2 vials
Ms IL-13 Antibody-Coated Wells, 96-well strip-well plate	1 plate
Ms IL-13 Biotin Conjugate; contains 8 mM sodium azide	11 mL
Extraction Solution	10 mL
Streptavidin-Peroxidase (HRP) (100X); contains 0.05% Proclin™ 300	0.125 mL
Streptavidin-Peroxidase (HRP) Diluent; contains 0.04% Proclin™ 300	25 mL
Wash Buffer Concentrate (25X)	100 mL
Stabilized Chromogen, Tetramethylbenzidine (TMB)	25 mL
Stop Solution	25 mL
Plate Covers, adhesive strips	4

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at or near 450 nm
- Plate washer–automated or manual (squirt bottle, manifold dispenser, or equivalent)
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solutions

Before you begin

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

- Review the **Procedural guidelines** and **Plate washing directions** in the *ELISA Technical Guide* available at **thermofisher.com**.
- Allow reagents to reach room temperature before use. Mix to redissolve any precipitated salts.

Prepare 1X Wash Buffer

1. Dilute 16 mL of Wash Buffer Concentrate (25X) with 384 mL of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 14 days.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera. If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Pre-dilute samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

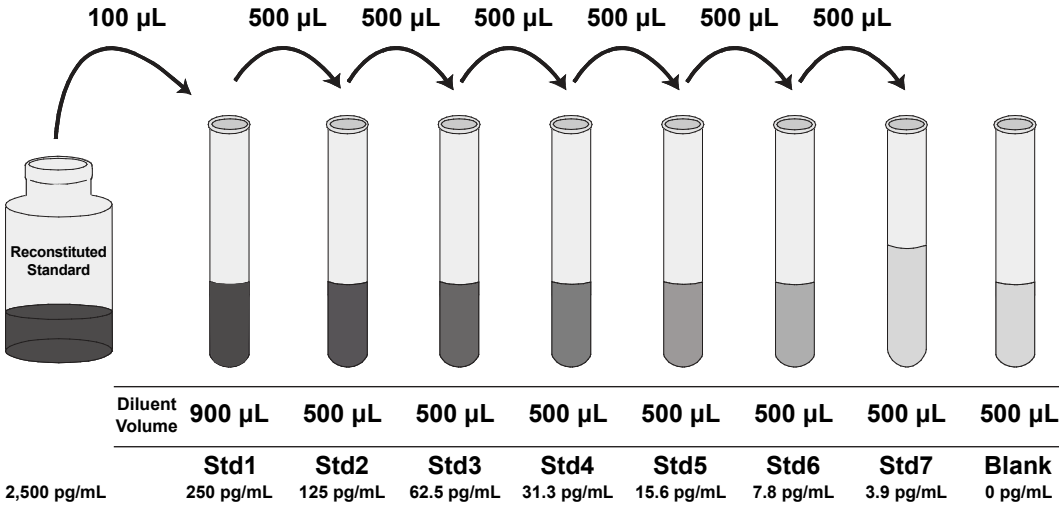
- Dilute samples >250 pg/mL with Standard Diluent Buffer.
- Dilute samples prepared in Extraction Solution 1:10 or greater in Standard Diluent Buffer (e.g., 10 µL sample into 90 µL buffer). This dilution is necessary to reduce the matrix effect of the Cell Extraction Buffer. SDS concentration should be less than 0.01% before adding to the plate. While a 1:10 sample dilution has been found to be satisfactory, higher dilutions such as 1:25 or 1:50 may be optimal.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

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

1. Reconstitute Ms IL-13 Standard to 2,500 pg/mL with Standard Diluent Buffer. Refer to the standard vial label for instructions. Swirl or mix gently and allow the contents to sit for 10 minutes to ensure complete reconstitution. Label as 2,500 pg/mL mouse IL-13. **Use the standard within 1 hour of reconstitution.**
2. Add 100 µL Reconstituted Standard to one tube containing 900 µL Standard Diluent Buffer and mix. Label as 250 pg/mL mouse IL-13.
3. Add 500 µL Standard Diluent Buffer to each of 7 tubes labeled as follows: 125, 62.5, 31.3, 15.6, 7.8, 3.9, and 0 pg/mL mouse IL-13.
4. Make serial dilutions of the standard as shown in the following dilution diagram. Mix thoroughly between steps.
5. Remaining reconstituted standard should be discarded or frozen in aliquots at –80°C for further use. Standard can be frozen and thawed one time only without loss of immunoreactivity.



Reconstitute controls

1. Add 1 mL distilled water to lyophilized controls. Refer to control vial labels for values and acceptable ranges in pg/mL.
2. Mix gently and allow to sit for 10 minutes.

   Note: After reconstitution, aliquot and store at ≤ –20°C. Avoid repeated freeze/thaw cycles. Do not extract controls.

Prepare 1X Streptavidin-HRP solution

Note: Prepare 1X Streptavidin-HRP within 15 minutes of usage.

The Streptavidin-HRP (100X) is in 50% glycerol, which is viscous. To ensure accurate dilution:

1. For each 8-well strip used in the assay, pipet 10 µL Streptavidin-HRP (100X) solution, wipe the pipette tip with clean absorbent paper to remove any excess solution, and dispense the solution into a tube containing 1 mL of Streptavidin-HRP Diluent. Mix thoroughly.
2. Return the unused Streptavidin-HRP (100X) solution to the refrigerator.

Perform ELISA (Total assay time: 4 hours)

IMPORTANT! Perform a standard curve with each assay.

- Allow all components to reach room temperature before use. Mix all liquid reagents prior to use.
- Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store at 2°C to 8°C for future use.

1 Bind antigen

a. Add 200 µL of standards, controls, or samples (see "Pre-dilute samples" on page 2) to the appropriate wells. Leave the wells for chromogen blanks empty.
b. Cover the plate with a plate cover and incubate for 2 hours at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

2 Add Biotin Conjugate

a. Add 100 µL Ms IL-13 Biotin Conjugate solution into each well except the chromogen blanks.
b. Cover the plate with plate cover and incubate for 1 hour at room temperature.
c. Thoroughly aspirate the solution and wash wells 4 times with 1X Wash Buffer.

3 Add Streptavidin-HRP

a. Add 100 µL 1X Streptavidin-HRP solution (see page 2) into each well except the chromogen blanks.
b. Cover the plate with a plate cover and incubate for 30 minutes at room temperature.
c. Thoroughly aspirate the solution from the wells and wash wells 4 times with 1X Wash Buffer.

4 Add Stabilized Chromogen

a. Add 100 µL Stabilized Chromogen to each well. The substrate solution begins to turn blue.
b. Incubate for 30 minutes at room temperature in the dark.

Note: TMB should not touch aluminum foil or other metals.

5 Add Stop Solution

Add 100 µL Stop Solution to each well. Tap the side of the plate to mix. The solution in the wells changes from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 2 hours after adding the Stop Solution.
2. Use curve-fitting software to generate the standard curve. A 4 parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns and controls, prior to plotting.
3. Read the concentrations for unknown samples and controls from the standard curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.

   Note: Dilute samples producing signals greater than the upper limit of the standard curve in Standard Diluent Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve example

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

Standard Mouse IL-13 (pg/mL)	Optical Density (450 nm)
250	3.10
125	1.87
62.5	1.12
31.3	0.61
15.6	0.39
7.8	0.23
3.9	0.15
0	0.07

Inter-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	12.98	47.67	154.90
Standard Deviation	1.44	3.09	11.50
% Coefficient of Variation	11.0	6.0	7.0

Intra-assay precision

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

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	12.37	46.20	150.50
Standard Deviation	0.98	2.49	10.10
% Coefficient of Variation	8.0	5.0	7.0

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

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

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

Sensitivity
The analytical sensitivity of the assay is <2 pg/mL mouse IL-13. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times.

Specificity
Buffered solutions of a panel of substances at 50 ng/mL were assayed with the Mouse IL-13 ELISA 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.

Limited product warranty
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Product label explanation of symbols and warnings

REF	Catalog Number	LOT	Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents

Manufacturer's address: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

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