Quantikine® ELISA

Mouse IL-13 Immunoassay

Catalog Number M1300CB
SM1300CB
PM1300CB

For the quantitative determination of mouse Interleukin 13 (IL-13) concentrations in cell culture supernates.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Mouse interleukin 13 (IL-13), previously designated P600, is a cytokine that is produced primarily by activated Th2 lymphocytes (1, 2). IL-13 shows approximately 30% amino acid (aa) sequence identity with IL-4 and shares many biological properties with IL-4. IL-13 has multiple effects on the differentiation and functions of monocytes/macrophages. It can suppress the cytotoxic functions of monocytes/macrophages as well as their production of proinflammatory cytokines. Although human or mouse IL-13 has effects similar to those of IL-4 on human B cells, mouse IL-13 has no effect on mouse B cells. Unlike IL-4, which is a growth and differentiation factor for human and mouse T cells, IL-13 has no effects on either human or mouse T cells (1, 2).

The sequence of mouse IL-13 cDNA predicts a 131 aa residue precursor protein containing a 20 aa residue signal peptide that is cleaved to form the mature protein (3). At the amino acid sequence level, mature mouse IL-13 is approximately 58% identical to human IL-13. While human and mouse IL-13 are equally active on human cells, human IL-13 is much less active than the mouse protein on mouse cells. The gene for IL-13 has been mapped to mouse chromosome 11, in close proximity to the genes for IL-3, IL-4, IL-5, and GM-CSF (1, 2, 4).

The biological effects of IL-13 are mediated by specific high-affinity cell surface receptor complexes. The functional IL-13 receptor complex has been shown to consist of the low-affinity IL-13 receptor α chain and the IL-4 receptor α subunit (IL-4 Rα) (5-7). This IL-13 receptor complex may also serve as an alternate high-affinity IL-4 receptor complex in IL-4 responsive cells that lack the γ_c chain (5, 6).

The Quantikine® Mouse IL-13 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-13 levels in cell culture supernates. It contains *E. coli*-expressed recombinant mouse IL-13 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse IL-13 accurately. Results obtained using natural mouse IL-13 showed dose curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-13.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse IL-13 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-13 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-13 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-13 bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the SDS on our website prior to use.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # M1300CB	CATALOG # SM1300CB	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-13 Microplate	890411	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse IL-13.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IL-13 Standard	890404	1 vial	3 vials	Recombinant mouse IL-13 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Mouse IL-13 Control	890228	1 vial	3 vials	Recombinant mouse IL-13 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse IL-13 Conjugate	892702	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse IL-13 conjugated to horseradish-peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-14	895180	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5T	895175	2 vials	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

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

M1300CB contains sufficient materials to run ELISAs on two 96 well plates.
SM1300CB (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM1300CB). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Test tubes for dilution of standards.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *This assay is only validated for cell culture supernate samples.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

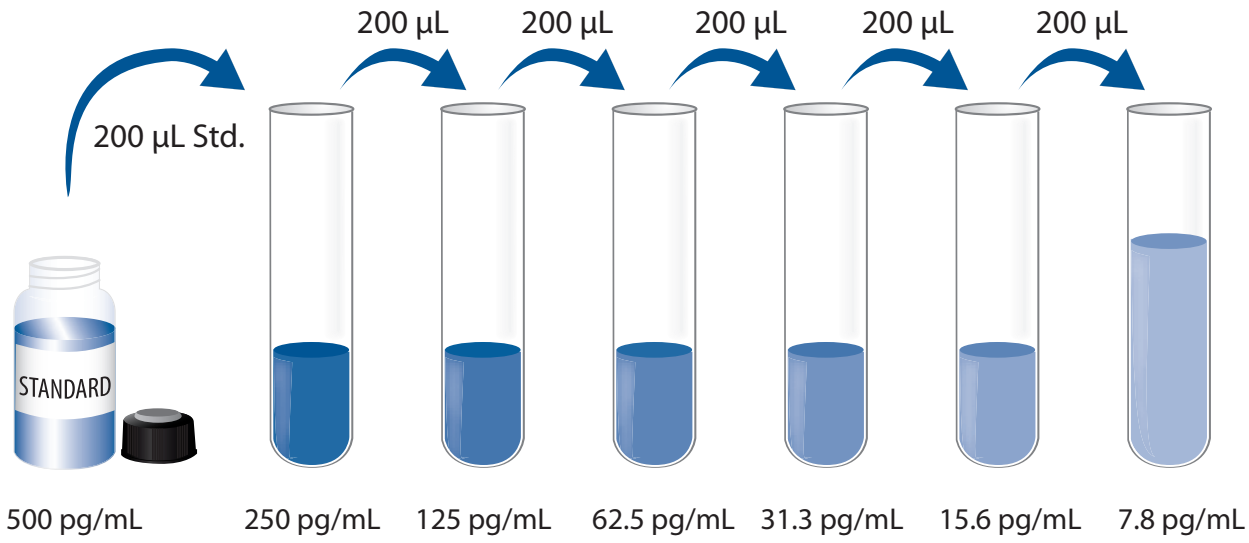
Mouse IL-13 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse IL-13 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-13 Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-13 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5T serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-14 to each well. *Assay Diluent RD1-14 may contain a precipitate even when mixed well before and during its use.*
4. Add 50 µL of standard, control, or sample to each well. Cover with the adhesive strip provided. Mix by gently tapping the plate frame for 1 minute. Incubate for 2 hours at room temperature.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. 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.
6. Add 100 µL of Mouse IL-13 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 4.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

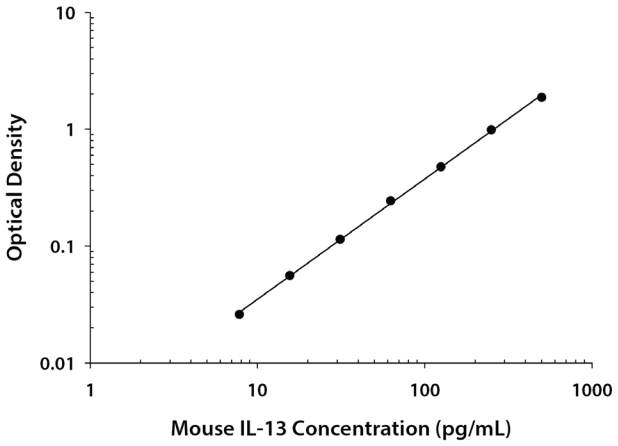
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the mouse IL-13 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.035 0.036	0.036	—
7.8	0.063 0.062	0.062	0.026
15.6	0.091 0.092	0.092	0.056
31.3	0.150 0.151	0.150	0.114
62.5	0.276 0.284	0.280	0.244
125	0.502 0.523	0.512	0.476
250	1.005 1.040	1.022	0.986
500	1.869 1.934	1.902	1.866

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	33.9	100	432	31.6	93.4	405
Standard deviation	1.1	2.7	11.2	2.5	7.2	17.7
CV (%)	3.2	2.7	2.6	7.9	7.7	4.4

RECOVERY

The recovery of mouse IL-13 spiked to levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=13)	99	89-119%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with mouse IL-13 were diluted with calibrator diluent and assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates (natural mouse IL-13)	Neat	407	———	———
	1:2	207	204	101
	1:4	101	102	99
	1:8	51	51	100
	1:16	23	26	88
Cell culture media (DMEM + 10% FBS) spiked with recombinant mouse IL-13	Spiked	286	———	———
	1:2	149	143	104
	1:4	72	72	100
	1:8	37	36	103
	1:16	18	18	100

SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-13 is typically less than 1.5 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse IL-13 produced at R&D Systems®.

SAMPLE VALUES

Cell Culture Supernates:

D10.G4.1 mouse helper T lymphocytes (1 x 10^5 cells/mL) were cultured 4 days in RPMI supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 10 ng/mL recombinant human IL-2, irradiated C3H spleen cells (3 x 10^5 cells/mL), and conalbumin (100 µg/mL). An aliquot of the cell culture supernate was removed, assayed for mouse IL-13, and measured 15 ng/mL.

EL-4 mouse lymphoblast cells (9 x 10^5 cells/mL) were cultured for 2 days in DMEM supplemented with 10% fetal bovine serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA. An aliquot of the cell culture supernate was removed, assayed for mouse IL-13, and measured 10 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-13.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse IL-13 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-7
IL-9
IL-10
IL-10 R
JE
KC
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant human:

IL-4
IL-4 Rα
IL-13

Recombinant rat IL-13 does not interfere but does cross-react approximately 0.1% in this assay.

REFERENCES

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