Quantikine® ELISA

Mouse IL-5 Immunoassay

Catalog Number M5000
SM5000
PM5000

For the quantitative determination of mouse Interleukin 5 (IL-5) concentrations in cell culture supernates and serum.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

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

Interleukin 5 (IL-5), also known as T-cell replacing factor, B-cell growth factor II, eosinophil differentiation factor, and eosinophil colony stimulating factor, is a pleiotropic cytokine produced primarily by T cells (1-4). It supports the proliferation and differentiation of mouse, but not human, B cells, and enhances IgM, IgG_1, IgA, and IgE secretion. IL-5 chemoattracts and enhances the survival and the effector functions of mature eosinophils and synergizes with various colony stimulating factors to increase eosinophil progenitor production and eosinophil expansion. Because of its diverse effects on eosinophils, IL-5 is strongly implicated in the pathogenesis of asthma and other hypereosinophilic inflammatory conditions (4-6).

Native mouse IL-5 is a disulfide-linked homodimeric 40-45 kDa glycoprotein with N- and O-linked carbohydrate chains. Mouse IL-5 cDNA encodes a 133 amino acid (aa) residue precursor protein containing a hydrophobic signal peptide that is cleaved to yield a 113 aa residue mature protein. Mature human IL-5 is approximately 70% identical at the amino acid level to mouse IL-5. Whereas mouse and human IL-5 are equally active on human cell lines, human IL-5 is much less active than mouse IL-5 in mouse cell assays (1-3).

The functional mouse IL-5 receptor is a heterodimer consisting of an α and a β subunit (1-3). Both subunits are type I membrane proteins belonging to the cytokine receptor superfamily. The α subunit binds IL-5 specifically with intermediate affinity. The β subunit, also known as AIC2B, is a common subunit (β_c) in the high-affinity receptor complexes of IL-3, IL-5 or GM-CSF. The β_c subunit does not bind IL-5 by itself but associates with the IL-5 Rα subunit to form the functional high-affinity IL-5 receptor. In addition to the membrane-bound form of mouse IL-5 Rα, soluble isoforms of IL-5 Rα encoded by alternatively spliced mRNAs have been identified (7). Purified soluble mouse IL-5 Rα has been shown to be an IL-5 antagonist. Mouse IL-5 Rα expression is limited to eosinophils, B cells and mast cells. The expression of the β_c subunit, however, is detected on various lineages of hematopoietic cells.

The Quantikine® Mouse IL-5 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-5 in cell culture supernates and serum. It contains *Sf* 21-expressed recombinant mouse IL-5 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate recombinant mouse IL-5. Results obtained using natural mouse IL-5 showed linear curves that were parallel to the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-5.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-5 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-5 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse IL-5 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-5 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 standard 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.
- It is recommended that samples be pipetted within 10 minutes.
- 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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between steps may improve assay precision.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # M5000	CATALOG # SM5000	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-5 Microplate	890665	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-5.	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-5 Standard	890666	1 vial	3 vials	Recombinant mouse IL-5 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.*
Mouse IL-5 Control	890668	1 vial	3 vials	Recombinant mouse IL-5 in a buffered protein base with preservatives; lyophilized. The assayed value of the control should be within the range specified on the label.	
Mouse IL-5 Conjugate	890667	1 vial	3 vials	23 mL/vial of a monoclonal antibody specific for mouse IL-5 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-38	895301	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD6-12	895214	1 vial	3 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.

M5000 contains sufficient materials to run ELISAs on two 96 well plates.
SM5000 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM5000). 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.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.

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 MSDS on our website prior to use.

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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Grossly hemolyzed or lipemic samples are not suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

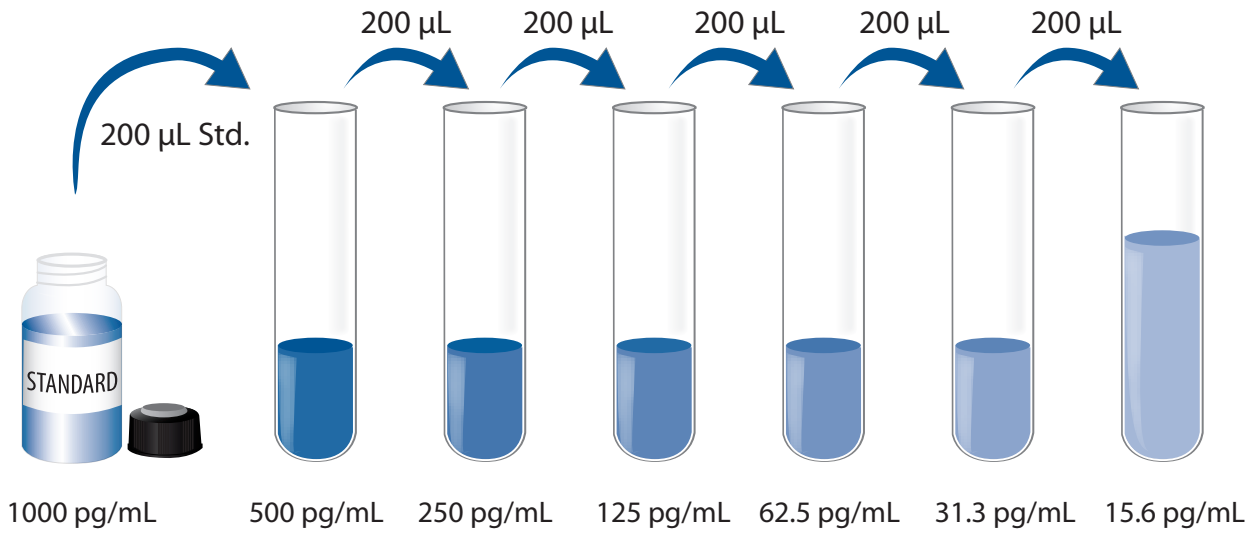
Mouse IL-5 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 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-5 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-5 Standard with Calibrator Diluent RD6-12. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 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 RD6-12 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-5 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD6-12 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 samples, standards, and control be assayed in duplicate.

1. Prepare reagents, samples, and standard dilutions as directed by 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 RD1-38 to each well.
4. Add 50 µL of standard, control, or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature.

   Note: *Samples, controls, and standards must be pipetted within 10 minutes.*
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-5 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 5.
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

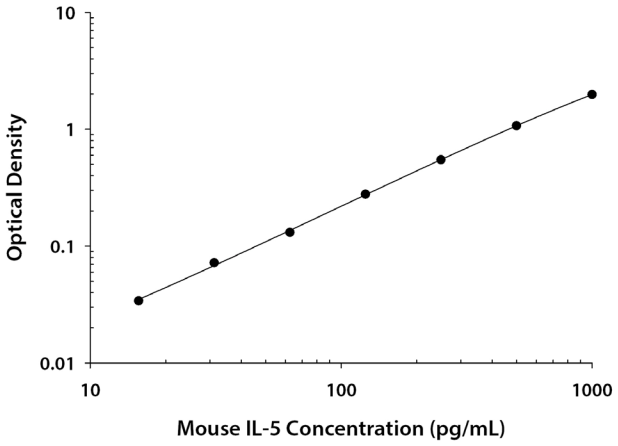
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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. The data may be linearized by plotting the log of the mouse IL-5 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.

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	0.038 0.039	0.038	—
15.6	0.073 0.071	0.072	0.034
31.3	0.113 0.107	0.110	0.072
62.5	0.170 0.168	0.169	0.131
125	0.328 0.305	0.316	0.278
250	0.598 0.575	0.586	0.548
500	1.117 1.098	1.108	1.070
1000	2.041 1.994	2.018	1.980

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 components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	41.5	117	428	43.4	123	447
Standard deviation	2.4	3.9	8.6	1.8	5.3	20.8
CV (%)	5.8	3.3	2.0	4.1	4.3	4.7

RECOVERY

The recovery of mouse IL-5 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=5)	99	85-110%
Serum (n=5)	92	81-104%

LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with various concentrations of mouse IL-5 in each matrix were diluted with calibrator diluent and then assayed.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}}$ x 100
Cell culture supernates	Neat	838	———	———
	1:2	405	419	97%
	1:4	203	210	97%
	1:8	104	105	99%
	1:16	48	52	92%
Serum	Spiked	765	———	———
	1:2	383	382	100%
	1:4	201	191	105%
	1:8	100	96	104%
	1:16	48	48	100%

SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-5 is typically less than 7 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 *Sf* 21-expressed recombinant mouse IL-5 produced at R&D Systems®.

SAMPLE VALUES

Serum - Forty samples were evaluated for detectable levels of mouse IL-5 in this assay. All samples read below the lowest standard, 15.6 pg/mL.

Cell Culture Supernates - 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 µg/mL PMA. An aliquot of the cell culture supernate was removed, assayed for mouse IL-5, and measured 40 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-5.

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-5 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
Eotaxin
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12
IL-13
IL-17
IL-18
JE/MCP-1
KC
Leptin
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
OSM
SCF
TNF-α
Tpo
VEGF

Recombinant human:

IL-5
IL-5 Rα

Recombinant rat IL-5 cross-reacts approximately 4.0% in this assay.

REFERENCES

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