Quantikine™ ELISA

Mouse IL-3 Immunoassay

Catalog Number M3000

For the quantitative determination of mouse Interleukin 3 (IL-3) concentrations in cell culture supernates and serum.

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 3 (IL-3) is a pleiotropic broad spectrum regulator of hematopoiesis that acts on numerous target cells, including primitive pluripotent hematopoietic stem cells, various lineage committed progenitors and mature hematopoietic cells (1-8). IL-3 has been shown to enhance the survival and stimulate the proliferation of hematopoietic stems cells and progenitor cells. IL-3 has also been shown to regulate the functional activity of mature mast cells, megakaryocytes, basophils, eosinophils and macrophages. Because of its multiple functions and targets, IL-3 was also known under different names, including mast cell growth factor, persisting (P)-cell-stimulating factor, multi-lineage colony-stimulating factor, burst-promoting activity, Thy-1-stimulating activity, histamine-producing cell stimulating activity, hematopoietic cell growth factor, multilineage hematopoietic growth factor, and CFUs stimulating factor.

Mouse IL-3 cDNA encodes a 166 amino acid (aa) residue precursor protein containing a 26 aa residue hydrophobic signal peptide (9-11). As a result of additional proteolytic cleavage beyond the predicted signal peptide, the N-terminal sequence of native mature IL-3 has been shown to contain either Ala 27 or Asp 33 (2). Mature rat and human IL-3 share approximately 59% and 29% aa sequence identity, respectively, with mouse IL-3 (2, 12). Consistent with this lack of aa sequence homology, mouse IL-3 is highly species-specific, exerting little or no activity on rat or human cells. IL-3 is produced primarily by activated T cells. In addition, activated mouse mast cells, mouse keratinocytes, neurons/astrocytes, and microglia cells have also been shown to produce IL-3 (1-3).

IL-3 exerts its biological activities through binding to specific cell surface receptors. The high affinity receptor responsible for IL-3 signaling is composed of an α and a β subunit (13, 14). The mouse IL-3 Rα is a member of the cytokine receptor superfamily and binds IL-3 with low affinity. Two distinct but homologous mouse β subunits, AIC2A (β_{IL-3}) and AIC2B (β_c) are known in the mouse. Both β_c and β_{IL-3} are also members of the cytokine receptor family. Either β_{IL-3} or β_c can combine with IL-3 Rα to form the functional high affinity mouse IL-3 receptor. In addition, β_c has been shown to be a common β subunit for mouse GM-CSF and IL-5 receptors (15-18). β_{IL-3} binds IL-3 with low affinity, whereas β_c does not bind any cytokines, including IL-3, IL-5 and GM-CSF. β_{IL-3} and β_c have been shown to co-express on IL-3-responsive cells (3, 19, 20). Both the α and β subunits of IL-3 R are present on bone marrow progenitor cells, various myeloid lineage cells, and on a portion of $CD19^+$ B cells (19, 20).

The Quantikine™ Mouse IL-3 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-3 in cell culture supernates and serum. It contains *E. coli*-expressed recombinant mouse IL-3 and antibodies raised against recombinant mouse IL-3. This Immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IL-3 showed dose response 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-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse IL-3 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-3 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-3 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 10 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-3 Microplate	890605	Two 96 well microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse IL-3.	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-3 Standard	890607	Recombinant mouse IL-3 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.*
Mouse IL-3 Control	890608	Recombinant mouse IL-3 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-3 Conjugate	890606	21 mL of a polyclonal antibody specific for mouse IL-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-12	895214	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	8 adhesive strips.	

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

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 SDS 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. 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 may not be suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

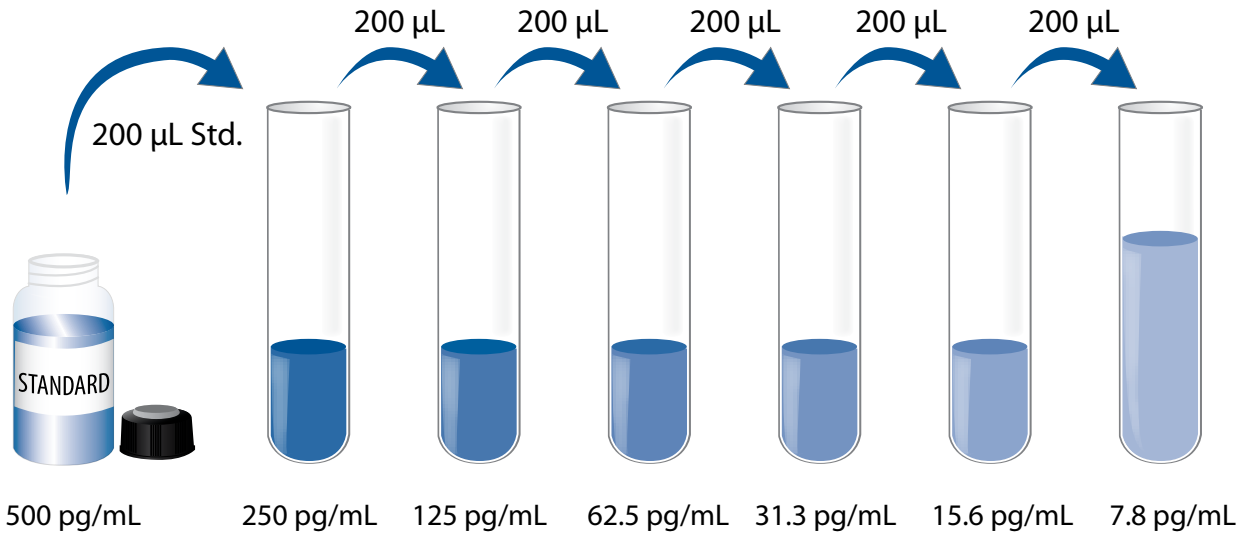
Mouse IL-3 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 of 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-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-3 Standard with Calibrator Diluent RD6-12. 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 RD6-12 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse IL-3 Standard (500 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 standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, 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 RD1W 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.
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 by inverting the plate and blotting it against clean paper towels.
6. Add 100 µL of Mouse IL-3 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

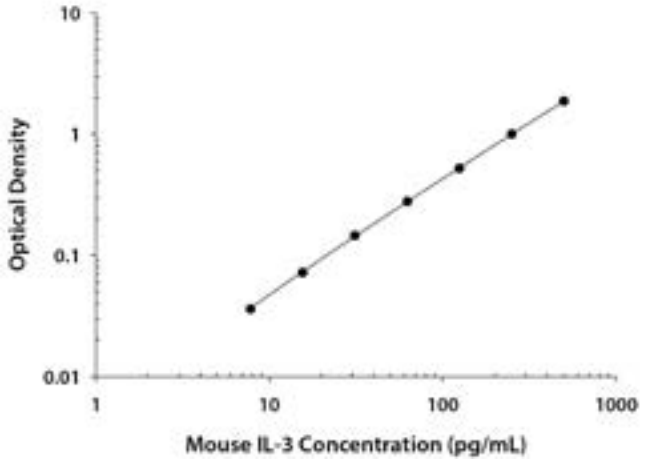
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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-3 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	0.025 0.027	0.026	—
7.8	0.061 0.062	0.062	0.036
15.6	0.095 0.101	0.098	0.072
31.3	0.162 0.181	0.172	0.146
62.5	0.286 0.324	0.305	0.279
125	0.528 0.571	0.550	0.524
250	0.989 1.066	1.028	1.002
500	1.895 1.903	1.899	1.873

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)	33	119	396	32	120	400
Standard deviation	1.7	8.3	22	2.5	5.5	18
CV (%)	5.2	7.0	5.6	7.8	4.6	4.5

RECOVERY

The recovery of mouse IL-3 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)	104	91-114%
Serum (n=5)	96	90-106%

LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with high concentrations of mouse IL-3 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Typical data are shown below.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Neat	364	———	———
	1:2	174	182	96
	1:4	89	91	98
	1:8	43	46	93
	1:16	20	23	87
Serum	Spiked	430	———	———
	1:2	207	215	96
	1:4	104	108	96
	1:8	53	54	98
	1:16	28	27	104

SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-3 was found to be < 2.5 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-3 produced at R&D Systems®.

The mouse IL-3 NIBSC/WHO reference standard 91/662, which was intended as a bioassay standard, was evaluated in this kit. Each ampule contains a nominal 1 µg of recombinant mouse IL-3, and was assigned an arbitrary unitage of 100,000 IU/ampule.

NIBSC/WHO 91/662 : 1 Unit of Standard = 6.5 pg of Quantikine™ Mouse IL-3.

SAMPLE VALUES

Serum - Forty samples were evaluated for detectable levels of mouse IL-3 in this assay. Thirty-eight samples read below the lowest standard, 7.8 pg/mL. Two samples read 24 and 26 pg/mL, respectively.

Cell Culture Supernates:
EL-4 mouse lymphoblast cells (9 x 10^5 cells/mL) were cultured for 2 days in DMEM plus 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-3, and measured 4.4 ng/mL.

WEHI-3 mouse myelomonocytic leukemia cells (5 x 10^5 cells/mL) were cultured for 2-4 days in RPMI plus 10% fetal bovine serum and L-glutamine. An aliquot of the cell culture supernate was removed, assayed for mouse IL-3, and measured 1.6 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-3.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse IL-3 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-2
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12
IL-13
KC
Leptin
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant human:

G-CSF
GM-CSF
IL-3
IL-3 Rα
IL-5
IL-5 Rα
M-CSF
SCF

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