Quantikine® ELISA

Mouse IL-23 Immunoassay

Catalog Number M2300

For the quantitative determination of mouse Interleukin 23 (IL-23) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please 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 23 (IL-23) is a heterodimeric cytokine that is related to IL-12 (1-3). It is composed of two disulfide-linked subunits, a 19 kDa (p19) subunit that is unique to IL-23, and a 40 kDa (p40, IL-12β) subunit that is shared with IL-12 (3-7). The mouse p19 cDNA encodes a 196 amino acid (aa) precursor with a putative 21 aa signal peptide and 175 aa mature protein with structural similarity to the IL-6 family of single chain cytokines and to the p35 subunit of IL-12. The mouse p40 cDNA encodes a 335 aa precursor with a putative 22 aa signal peptide and 313 aa mature protein that contains four potential glycosylation sites, a C2-type immunoglobulin domain and a fibronectin type III domain. Mature mouse p19 and p40 share 88% and 92% aa sequence identity, respectively, with the corresponding rat subunits. IL-23 is produced by activated macrophages, microglia, and monocyte-derived dendritic cells in response to pathogens including certain bacteria and viruses and/or their components (3, 6). The p19 subunit is also expressed by polarized T cells, but these cells do not express the p40 subunit (6).

The functional IL-23 receptor complex consists of two receptor subunits, the IL-12 receptor β1 subunit (IL-12 Rβ1) and the IL-23-specific receptor subunit (IL-23 R) (7). IL-12 Rβ1 is a 738 aa type I transmembrane glycoprotein with a 546 aa extracellular domain that contains a WSXWS motif and five fibronectin type III domains. IL-23 R is a 644 aa type I transmembrane glycoprotein with a 351 aa extracellular domain that contains two fibronectin type III domains. The IL-23 receptor complex is expressed in mouse Th1 and Th2 cells, bone marrow dendritic cells, IFN-γ-activated macrophages, and $CD4^+$ $CD45RB^{low}$ memory T cells (7). IL-23 initiates a signal transduction cascade similar to that of IL-12, and involves Jak2, Tyk2, STAT1, STAT3, STAT4, and STAT5 (7). IL-23 will bind to IL-12 Rβ1, but signaling requires the presence of the IL-23 R subunit (1-3).

IL-23 and IL-12 have overlapping and distinct biological activities. The IL-23 immune pathway induces the earliest recruitment of neutrophils to the site of infection, while the more classic host defense and cytotoxic response is stimulated by IL-12 (4). IL-12 drives the development of Th1 cells and induces production of IFN-γ by NK cells (3). In contrast, IL-23 has a role in the development and maintenance of a T cell subset, designated Th17, that is characterized by the production of IL-17A, IL-17F, IL-6, and TNF-α (3, 4, 8). The induction of Th17 cells involves the actions of TGF-β, while their survival and expansion appears to be IL-23-dependent (9-11). The IL-23/IL-17 axis is an important mediator of inflammation. In mouse models, transgenic over-expression of IL-23 leads to a lethal systemic inflammatory response (12). IL-23 effects on Th17 cells may also enhance the development of several models of autoimmune disease including experimental allergic encephalomyelitis (EAE), collagen-induced arthritis (CIA), colitis, and diabetes (5, 8, 13-17). IL-23 may also play a role in increased tumor growth associated with chronic inflammation (18). In humans, IL-23 has been found upregulated in several pathologies with dysregulated immune function including psoriasis, Crohn's disease, and multiple sclerosis (19-21).

The Quantikine® Mouse IL-23 immunoassay is a 4.5 hour solid-phase ELISA designed to measure IL-23 in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant mouse IL-23 and antibodies raised against the recombinant p19 and p40 subunits. This immunoassay has been shown to accurately quantitate recombinant mouse IL-23. Results obtained using natural mouse IL-23 showed dose response curves that were parallel to the standard curve obtained using the Quantikine® mouse kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-23.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-23 p19 subunit has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-23 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-23 p40 subunit 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 mouse IL-23 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, 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.
- 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-23 Microplate	893329	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-23 p19 subunit.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IL-23 Conjugate	893330	12 mL of a polyclonal antibody specific for mouse IL-23 p40 subunit conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse IL-23 Standard	893331	Recombinant mouse IL-23 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse IL-23 Control	893332	Recombinant mouse IL-23 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-14	895180	12 mL of a buffered protein solution with preservatives. *May contain a precipitate. Mix well before and during use.*	
Calibrator Diluent RD5-3	895436	21 mL of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	21 mL 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	4 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards and samples.

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.

Plasma - Collect plasma using heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *EDTA plasma is not recommended for use in this assay.*
Citrate plasma has not been validated for use in this assay.
Hemolyzed and icteric samples are not suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

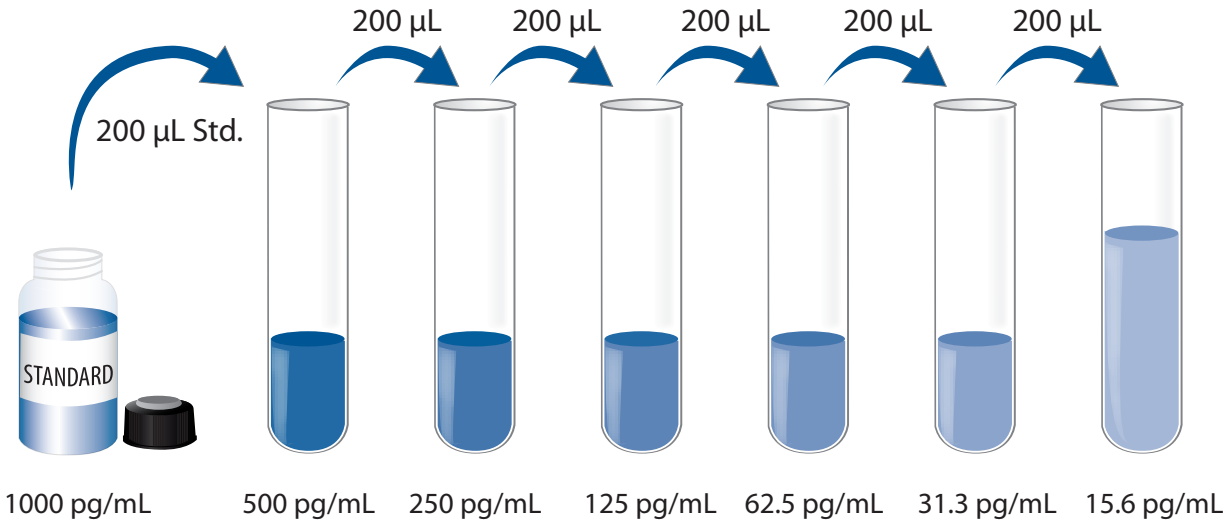
Mouse IL-23 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of 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-23 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-23 Standard with Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-3 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-23 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD5-3 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, control, and standards 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 RD1-14 to each well. *Assay Diluent RD1-14 may contain undissolved material even when mixed well before and during use.*
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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-23 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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

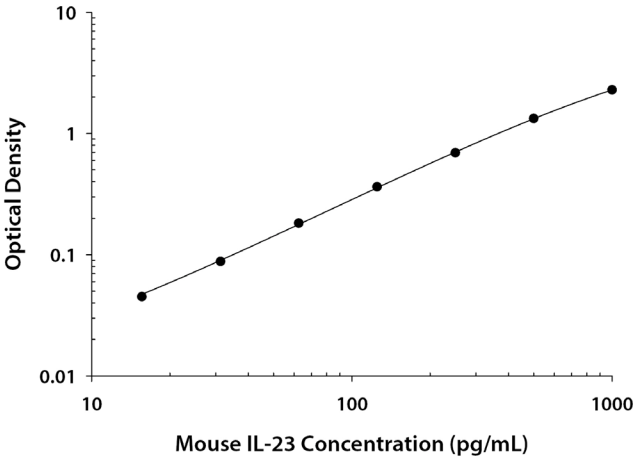
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D).

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-23 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 prior to assay, 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	0.049 0.049	0.049	———
15.6	0.091 0.097	0.094	0.045
31.3	0.137 0.137	0.137	0.088
62.5	0.223 0.238	0.231	0.182
125	0.409 0.415	0.412	0.363
250	0.734 0.749	0.742	0.693
500	1.367 1.389	1.378	1.329
1000	2.299 2.377	2.338	2.289

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 forty 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	40	40	40
Mean (pg/mL)	49.3	107	344	50.4	116	333
Standard deviation	3.72	5.84	22.7	3.69	7.14	18.6
CV (%)	7.5	5.5	6.6	7.3	6.2	5.6

RECOVERY

The recovery of mouse IL-23 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	103	99-108%
Serum (n=4)	95	90-101%
Heparin plasma (n=4)	99	88-117%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse IL-23 in each matrix were diluted with calibrator diluent and assayed.

		Cell culture supernates (n=4)	Serum (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	98	104	102
	Range (%)	93-101	102-105	97-106
1:4	Average % of Expected	98	107	109
	Range (%)	93-102	104-109	104-115
1:8	Average % of Expected	94	107	110
	Range (%)	88-98	100-110	105-115
1:16	Average % of Expected	93	108	110
	Range (%)	85-97	106-110	105-116

SENSITIVITY

Forty assays were evaluated and the minimum detectable dose (MDD) of mouse IL-23 ranged from 0.68-4.17 pg/mL. The mean MDD was 2.28 pg/mL.

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

CALIBRATION

This immunoassay is calibrated against highly purified *Sf* 21-expressed covalently-linked p19 and p40 subunits of recombinant mouse IL-23 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of mouse IL-23 in this assay. No detectable levels were observed.

Cell Culture Supernates - J774A.1 mouse reticulum cell sarcoma macrophage cells were cultured for three days in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 25 ng/mL lipopolysaccharide (LPS), and 100 ng/mL recombinant mouse GM-CSF. An aliquot of the cell culture supernate was removed, assayed for mouse IL-23, and measured 58.6 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-23.

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-23 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:	Recombinant rat:	Recombinant human:	Other recombinants:
IL-12	IL-12	IL-23	canine IL-12
IL-12 p35	IL-12 p35	IL-23 p19	porcine IL-12
IL-12 p40 dimer	IL-23	IL-23 R	canine IL-12 p40
IL-12 p40 monomer			
IL-12 Rβ			
IL-23 R			

This assay does not recognize free p19 or p40 subunits.

Recombinant mouse IL-23 p19 interferes at concentrations ≥ 50 ng/mL in this assay.

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