ELISA Kit
Catalog # KMC0231

Mouse
IL-23

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PURPOSE

The Invitrogen Mouse Interleukin-23 (Ms IL-23) ELISA is to be used for the quantitative determination of Ms IL-23 in serum, plasma, buffered solution and tissue culture medium. The assay will recognize both natural and recombinant Ms IL-23. This assay will not recognize the monomeric forms of the p19 or p40 subunits.

INTRODUCTION

IL-23 is a recently discovered cytokine that plays a role in the maturation of memory T cells. As with the closely related cytokines IL-12 and IL-27, IL-23 is a disulfide-linked heterodimeric protein, with a molecular mass of approximately 70 kDa. IL-23's two subunits are designated p19 and p40. A search of protein sequence data bases for proteins bearing homology with IL-6 led to the discovery of the p19 subunit. In addition to IL-6, the p19 subunit also bears homology with G-CSF and the p35 subunit of IL-12. The p40 subunit, which is common to both IL-23 and IL-12, shares homology with the receptors for IL-6 and ciliary neurotrophic factor. The expression of the p19 and the p40 subunits is differentially regulated. The orchestrated production of both subunits is required for the production of the biologically active cytokine.

The p19 subunit, produced by activated dendritic cells, macrophages, and endothelial cells, is upregulated in response to bacterial products that signal through Toll like receptor-2. Studies with $p19^{-/-}$ mice provide evidence for the role of IL-23 in the generation of memory T cell-dependent humoral and cell-mediated immune responses to antigen.

The p40 subunit is produced by activated dendritic cells, macrophages/ monocytes, microglia of the brain, bone marrow cells cultured in the presence of stem cell factor (c-kit ligand), and keratinocytes. The p40

subunit is highly inducible and under the control of several transcription factors, such as IRF-1, c-Rel, Ets-2, and Ets-related components, as well as interferon consensus sequence-binding protein (ICSBP).

The receptor for IL-23 is composed of IL-12Rb1 (the IL-12 p40 subunit receptor), plus an IL-23-specific receptor chain. IL-12Rb2, a second component of the IL-12 receptor, is incapable of binding IL-23. The IL-23 receptor constitutively associates with JAK2. In response to ligand stimulation, the receptor/JAK2 complex stimulates the phosphorylation and activation of STAT4. Other signaling molecules involved in IL-23 signaling include TYK2, STAT1, STAT3, and STAT5.

Several cell types respond to stimulation by IL-23. IL-23 induces a strong proliferative response with memory T cells (defined by $CD4^+$ $CD45b^{low}$); with this cell type, as well as PHA blast T cells, IL-23 induces interferon-γ production. When applied to peritoneal macrophages, IL-23 stimulates the production of IL-1 and tumor-necrosis factor (TNF), suggesting a role as an autocrine inflammatory cytokine with these cells.

The role of IL-23 in the pathogenesis of autoimmune inflammatory diseases is currently under investigation. Recent studies have shown that IL-23 promotes the development of a pathogenic $CD4^+$ T-cell population that produces IL-6, IL-17 and TNF, as well as other proinflammatory cytokines. In a mouse model of autoimmune disease, experimental autoimmune encephalopathy (EAE), administration of neutralizing antibodies directed to IL-17 abrogate the autoimmune damage mediated by the IL-23 expanded population, suggesting an important cytokine cascade involving IL-23 and IL-17 in autoimmune inflammatory diseases.

For Research Use Only. CAUTION: Not for human or animal therapeutic or diagnostic use.

Read entire protocol before use.

PRINCIPLE OF THE METHOD

The Invitrogen Ms IL-23 kit is a solid phase sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). A highly purified antibody against Ms IL-23p19 subunit has been coated onto the wells of the microtiter strips provided.

During the first incubation, standards of known Ms IL-23 content, controls, and unknown samples are pipetted into the coated wells. After washing, a biotinylated anti-IL-12/IL-23p40 monoclonal detection antibody, is added.

After washing, Streptavidin-Peroxidase (enzyme) is added. This binds to the biotinylated antibody to complete the four-member sandwich. After a third incubation and washing to remove all the unbound enzyme, a substrate solution is added, which is acted upon by the bound enzyme to produce color. The intensity of this colored product is directly proportional to the concentration of Ms IL-23 present in the original specimen.

REAGENTS PROVIDED

Note: *Store all reagents at 2 to 8°C.*

Reagent	***96 Test Kit***
Ms IL-23 Standard, recombinant Ms IL-23. Contains 0.1% sodium azide. Refer to vial label for quantity and reconstitution volume.	2 vials
Standard Diluent Buffer. Contains 0.1% sodium azide; 25 mL per bottle.	1 bottle
Incubation Buffer. Contains 0.05% sodium azide; 12 mL per bottle.	1 bottle
Ms IL-23 High and Low Controls, recombinant Ms IL-23 lyophilized. Contains 0.1% sodium azide. Refer to vial label for reconstitution volume and range.	2 vials
Ms IL-23 Antibody Coated Wells. 96 Well Plate.	1 plate
Ms IL-23 Biotin Conjugate, (Biotin-labeled anti-IL-12/IL-23p40). Contains 0.1% sodium azide; 11 mL per bottle.	1 bottle
Streptavidin-HRP (100X). Contains 3.3 mM thymol; 0.125 mL per vial.	1 vial
Streptavidin-HRP Diluent. Contains 0.1% Proclin® 300; 25 mL per bottle.	1 bottle
Wash Buffer Concentrate (25X). 100 mL per bottle.	1 bottle
Stabilized Chromogen, *Tetramethylbenzidine (TMB).* 25 mL per bottle.	1 bottle
Stop Solution; 25 mL per bottle.	1 bottle
Plate Covers, adhesive strips.	4

Disposal Note: 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.

SUPPLIES REQUIRED BUT NOT PROVIDED

1. Microtiter plate reader capable of measurement at or near 450 nm.
2. Calibrated adjustable precision pipettes, preferably with disposable plastic tips. (A manifold multi-channel pipette is desirable for large assays.)
3. Distilled or deionized water.
4. Plate washer: automated or manual (squirt bottle, manifold dispenser, etc.).
5. Data analysis and graphing software. Graph paper: linear (Cartesian), log-log, or semi-log, as desired.
6. Glass or plastic tubes for diluting and aliquoting standard.
7. Absorbent paper towels.
8. Calibrated beakers and graduated cylinders in various sizes.

PROCEDURAL NOTES/LAB QUALITY CONTROL

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.
2. **Microtiter plates should be allowed to come to room temperature before opening the foil bags.** Once the desired number of strips has been removed, immediately reseal the bag and store at 2 to 8°C to maintain plate integrity.

3. Samples should be collected in pyrogen/endotoxin-free tubes.
4. Samples should be frozen if not analyzed shortly after collection. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well prior to analysis.
5. When possible, avoid use of badly hemolyzed or lipemic serum and plasma samples. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
6. It is recommended that all standards, controls and samples be run in duplicate.
7. Serum, plasma, or tissue culture sample(s) that measure >500 pg/mL require additional dilution steps in *Standard Diluent Buffer*.
8. When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
9. Cover or cap all reagents when not in use.
10. **Do not mix or interchange different reagent lots from various kit lots**.
11. Do not use reagents after the kit expiration date.
12. Read absorbances within 30 minutes of assay completion.
13. The controls provided should be run with every assay. If control values fall outside pre-established ranges, the accuracy of the assay is suspect.
14. All residual wash liquid must be drained from the wells by efficient aspiration or by decantation followed by tapping the plate forcefully on absorbent paper. ***Never*** insert absorbent paper directly into the wells.
15. Because *Stabilized Chromogen* is light sensitive, avoid prolonged exposure to light. Also avoid contact between *Stabilized Chromogen* and metal, or color may develop.

SAFETY

All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.

DIRECTIONS FOR WASHING

Incomplete washing will adversely affect the test outcome. All washing must be performed with the *Wash Buffer Concentrate (25X)* provided.

Washing can be performed manually as follows: completely aspirate the liquid from all wells by gently lowering an aspiration tip (aspiration device) into the bottom of each well. Take care not to scratch the inside of the well.

After aspiration, fill the wells with at least 0.4 mL of diluted wash solution. Let soak for 15 to 30 seconds, then aspirate the liquid. Repeat as directed under **ASSAY METHOD**. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

Alternatively, the wash solution may be put into a squirt bottle. If a squirt bottle is used, flood the plate with wash buffer, completely filling all wells. After the washing procedure, the plate is inverted and tapped dry on absorbent tissue.

If using an automated washer, the operating instructions for washing equipment should be carefully followed.

SAMPLE PREPARATION

Mouse serum, plasma, tissue culture supernatant and controls, require a 2-fold dilution in the *Standard Diluent Buffer*. For example, add 110 µL of the sample to a clean microfuge tube, followed by 110 µL of *Standard Diluent Buffer*. Mix Well.

REAGENT PREPARATION AND STORAGE

A. Reconstitution and Dilution of Ms IL-23 Standard

The *Ms IL-23 Standard* is prepared from a highly purified baculovirus-expressed recombinant protein, produced by co-expression of the Mouse p19 and p40 subunits.

Note: Either glass or plastic tubes may be used for standard dilutions.

1. Reconstitute standard to 500 pg/mL with *Standard Diluent Buffer*. Refer to the standard vial label for instructions. Swirl or mix gently and allow to sit for 10 minutes to ensure complete reconstitution. Use the standard within 15 minutes of reconstitution.
2. Add 0.300 mL of *Standard Diluent Buffer* to each of 6 tubes labeled 250, 125, 62.5, 31.3, 15.6, and 7.8 pg/mL Ms IL-23.
3. Make serial dilutions of the standard as described in the following dilution table. Mix thoroughly between steps.

B. Dilution of Ms IL-23 Standard

Standard:	Add:	Into:
500 pg/mL	Prepare as described in Step 1.	
250 pg/mL	0.300 mL of the 500 pg/mL std.	0.300 mL of the Diluent Buffer
125 pg/mL	0.300 mL of the 250 pg/mL std.	0.300 mL of the Diluent Buffer
62.5 pg/mL	0.300 mL of the 125 pg/mL std.	0.300 mL of the Diluent Buffer
31.3 pg/mL	0.300 mL of the 62.5 pg/mL std.	0.300 mL of the Diluent Buffer
15.6 pg/mL	0.300 mL of the 31.3 pg/mL std.	0.300 mL of the Diluent Buffer
7.8 pg/mL	0.300 mL of the 15.6 pg/mL std.	0.300 mL of the Diluent Buffer
0 pg/mL	0.300 mL of the Diluent Buffer	An empty tube

Discard all remaining reconstituted and diluted standards after completing assay. Return the *Standard Diluent Buffer* to the refrigerator.

C. Storage and Final Dilution of Streptavidin-HRP (100X)

Please Note: The *Streptavidin-HRP (100X)* is in 50% glycerol. This solution is viscous. To ensure accurate dilution, allow *Streptavidin-HRP (100X)* to reach room temperature. Gently mix. Pipette *Streptavidin-HRP (100X)* slowly. Remove excess concentrate solution from pipette tip by gently wiping with clean absorbent paper.

1. Within 15 minutes of use, dilute 10 µL of this 100X concentrated solution with 1 mL of *Streptavidin-HRP Diluent* for each 8-well strip used in the assay. Label as Streptavidin-HRP Working Solution.

For Example:

# of 8-Well Strips	Volume of Streptavidin-HRP (100X)	Volume of Diluent
2	20 µL solution	2 mL
4	40 µL solution	4 mL
6	60 µL solution	6 mL
8	80 µL solution	8 mL
10	100 µL solution	10 mL
12	120 µL solution	12 mL

2. Return the unused *Streptavidin-HRP (100X)* to the refrigerator.

D. Dilution of Wash Buffer

Allow the *Wash Buffer Concentrate (25X)* to reach room temperature and mix to ensure that any precipitated salts have redissolved. Dilute 1 volume of the *Wash Buffer Concentrate (25X)* with 24 volumes of deionized water (e.g., 50 mL may be diluted up to 1.25 liters, 100 mL may be diluted up to 2.5 liters). Label as Working Wash Buffer.

Store both the concentrate and the Working Wash Buffer in the refrigerator. The diluted buffer should be used within 14 days.

ASSAY METHOD: PROCEDURE AND CALCULATIONS

Be sure to read the *Procedural Notes/Lab Quality Control* section before carrying out the assay.

Allow all reagents to reach room temperature before use. Gently mix all liquid reagents prior to use.

Note: A standard curve must be run with each assay.

1. Determine the number of 8-well strips needed for the assay. Insert these in the frame(s) for current use. (Re-bag extra strips and frame. Store these in the refrigerator for future use.)
2. Add 50 µL *Incubation Buffer* to all wells.
3. Add 100 µL of the *Standard Diluent Buffer* to the zero standard wells. Well(s) reserved for chromogen blank should be left empty.
4. Add 100 µL of standards to the appropriate microtiter wells. For all samples (serum, plasma, tissue culture supernatant and controls), add 50 µL of *Standard Diluent Buffer* to each well following the 50 µL of sample. Tap gently on the side of the plate for 30 seconds to mix.
5. Cover plate with *plate cover* and incubate for **2 hours at room temperature**.

6. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
7. Add 100 µL of *Ms IL-23 Biotin Conjugate* into each well except the chromogen blank(s). Tap on the side of the plate for 30 seconds to mix.
8. Cover plate with *plate cover* and incubate for **1 hour at room temperature**.
9. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
10. Add 100 µL Streptavidin-HRP Working Solution to each well except the chromogen blank(s). (Prepare the working dilution as described in **REAGENT PREPARATION AND STORAGE**, Section C.)
11. Cover plate with the *plate cover* and incubate for **30 minutes at room temperature**.
12. Thoroughly aspirate or decant solution from wells and discard the liquid. Wash wells 4 times. See **DIRECTIONS FOR WASHING**.
13. Add 100 µL of *Stabilized Chromogen* to each well. The liquid in the wells will begin to turn blue.
14. Incubate for **30 minutes at room temperature and in the dark**. ***Please Note*: Do not cover the plate with aluminum foil or metalized mylar**. The incubation time for chromogen substrate is often determined by the microtiter plate reader used. Many plate readers have the capacity to record a maximum optical density (O.D.) of 2.0. The O.D. values should be monitored and the substrate reaction stopped before the O.D. of the positive wells

exceed the limits of the instrument. The O.D. values at 450 nm can only be read after the *Stop Solution* has been added to each well. If using a reader that records only to 2.0 O.D., stopping the assay after 20 to 25 minutes is suggested.

15. Add 100 µL of *Stop Solution* to each well. Tap side of plate gently to mix. The solution in the wells should change from blue to yellow.
16. Read the absorbance of each well at 450 nm having blanked the plate reader against a chromogen blank composed of 100 µL each of *Stabilized Chromogen* and *Stop Solution*. Read the plate within 30 minutes after adding the *Stop Solution*.
17. Plot on graph paper the absorbance of the standards against the standard concentration. (Optimally, the background absorbance may be subtracted from *all* data points, including standards, unknowns and controls, prior to plotting.) Draw the best smooth curve through these points to construct the standard curve. If using curve fitting software, the four parameter algorithm provides the best curve fit.
18. Read the Ms IL-23 concentrations for unknown samples and controls from the standard curve plotted in step 17. **Multiply value(s) obtained for all samples by 2 to correct for the overall 1:2 dilution in step 4.** Samples producing signals greater than that of the highest standard (500 pg/mL) should be further diluted in the *Standard Diluent Buffer* and reanalyzed. Multiply the concentration found by the appropriate dilution factor.

TYPICAL DATA

The following data were obtained for the various standards over the range of 0 to 500 pg/mL Ms IL-23.

Standard Ms IL-23 (pg/mL)	Optical Density (450 nm)
0	0.075
	0.073
7.8	0.131
	0.128
15.6	0.189
	0.189
31.3	0.310
	0.307
62.5	0.547
	0.545
125	1.014
	1.005
250	1.805
	1.800
500	2.993
	3.008

LIMITATIONS OF THE PROCEDURE

Do not extrapolate the standard curve beyond the 500 pg/mL standard point; the dose-response is non-linear in this region and accuracy is difficult to obtain. Dilute samples with concentrations exceeding the linear portion of the Standard Curve with the appropriate diluent buffer; reanalyze these and multiply results by the appropriate dilution factor.

The influence of various drugs and the use of biological fluids in place of tissue culture media have not been thoroughly investigated. The rate of degradation of native Ms IL-23 in various matrices has not been investigated. The immunoassay literature contains frequent references to aberrant signals seen with some sera, attributed to heterophilic antibodies. Though such samples have not been seen to date, the possibility of this occurrence cannot be excluded.

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PERFORMANCE CHARACTERISTICS

SENSITIVITY

The minimum detectable dose of Ms IL-23 is <3 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 30 times, and calculating the corresponding concentration.

PRECISION

1. Intra-Assay Precision

Samples of known Ms IL-23 concentrations were assayed in replicates of 16 to determine precision within an assay.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	30	120.3	236.7
SD	1.3	5.0	11.3
%CV	4.3	4.1	4.8

SD = Standard Deviation
CV = Coefficient of Variation

2. Inter-Assay Precision

Samples were assayed 48 times in multiple assays to determine precision between assays.

	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	28.1	108	243
SD	2.5	8.8	15.5
%CV	8.9	8.1	6.4

SD = Standard Deviation
CV = Coefficient of Variation

LINEARITY OF DILUTION

Mouse serum, EDTA plasma, citrate plasma, and tissue culture medium spiked with recombinant Ms IL-23 were serially diluted in *Standard Diluent Buffer* over the range of the assay. Linear regression analysis of samples versus the expected concentration yielded average correlation coefficients of 0.9992 for serum, 0.9995 for EDTA plasma, 0.9988 for citrate plasma, and 0.9998 for tissue culture medium.

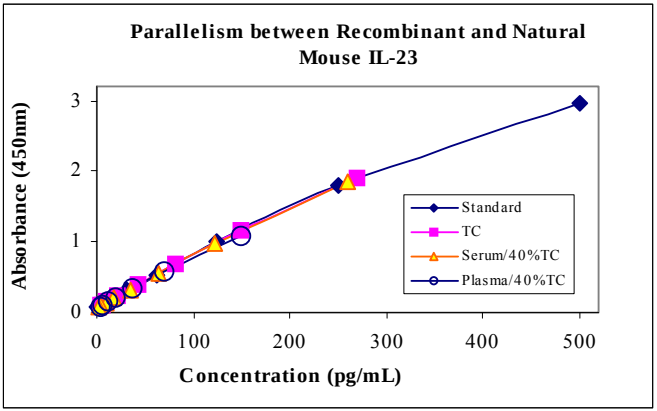
RECOVERY

The recoveries of recombinant Ms IL-23 added to mouse serum, EDTA plasma, citrate plasma, and tissue culture medium containing 10% fetal bovine serum or 10% calf serum were measured with the Invitrogen Ms IL-23 ELISA.

Sample Type	Average % Recovery
Serum*	96
EDTA plasma*	90
Citrate plasma*	80
RPMI+10% fetal bovine serum*	97
DMEM+10% calf serum*	95
***All samples were pre-diluted 2-fold in *Standard Diluent Buffer*.**	

PARALLELISM

Random mouse serum, plasma, and tissue culture supernatant were serially diluted in the *Standard Diluent Buffer*. The optical density of each dilution was plotted against the Ms IL-23 standard curve. Parallelism was demonstrated by the figure below and indicated that the standard accurately reflects the Ms IL-23 content in natural samples.



SPECIFICITY

Buffered solutions of a panel of substances ranging in concentrations from 0.3 to 56 ng/mL were assayed with the Invitrogen Ms IL-23 kit and found to have no cross-reactivity: Human DR-5, IFN-γ, IL-1β, IL-2R, IL-5, IL-6, IL-7, IL-10, IL-13, IL-15, IL-23, TNF-α, TNF-RI, TNF-RII, VEGF; Rat GM-CSF, IFN-γ, IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12, IL-13, IL-23, TNF-α, MIP-2, RANTES; Mouse Eotaxin, GM-CSF, IFN-γ, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-12/IL-23p40, IL-23p19, KC, MCP-1, and TNF-α.

HIGH DOSE HOOK EFFECT

No hook effect was observed with concentrations up to 1 μg/mL.

EXPECTED VALUES

Twenty-six normal serum and plasma samples, and forty-two cell culture supernatants were evaluated for the presence of Ms IL-23 in this assay.

Sample	Range (pg/mL)
Serum (n=15)	0
EDTA plasma (n=5)	0
Citrate plasma (n=6)	0 - 12.5
Cell culture supernatants J774 A.1 cells unstimulated	0
Cell culture supernatants J774 A1 cells stimulated	120 - 2330

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Explanation of symbols

Symbol	Description	Symbol	Description
REF	Catalogue Number	LOT	Batch code
RUO	Research Use Only	IVD	*In vitro* diagnostic medical device
	Use by		Temperature limitation
	Manufacturer	EC REP	European Community authorised representative
[-]	Without, does not contain	[+]	With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

Mouse IL-23 Assay Summary