Quantikine® ELISA

Mouse IL-12/IL-23 p40 Non Allele-specific Immunoassay

Catalog Number MP400
SMP400
PMP400

For the quantitative determination of total mouse Interleukin 12/23 p40 (IL-12/IL-23 p40) concentrations in cell culture supernates, serum, and plasma.*

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

Polymorphisms exist in the mouse IL-12/IL-23 p40 sequence. The monoclonal capture antibody used in this kit is not allele-specific. It recognizes IL-12/IL-23 p40 variants represented in mouse strains 129/J, AKR, A.SW, BALB/c, C3H, C57BL/6, DBA/1, DBA/2, NOD, NOR, NZB, NZC, NZO, and SJL (Ymer, S.I. et al. (2002) Genes and Immunity **3:151). An allele-specific mouse IL-12/23 p40 ELISA is also available (R&D Systems, Catalog # M1240).*

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 12 (IL-12) and Interleukin 23 (IL-23) are secreted heterodimeric glycoproteins belonging to the IL-12 cytokine family (1-4). The two cytokines share a common p40 (40 kDa) subunit, which is disulfide-linked with the p35 (35 kDa) subunit in IL-12, and with the p19 (19 kDa) subunit in IL-23. In addition to being secreted as a component of IL-12 or IL-23, free p40 monomers and homodimers are also secreted (3, 4). Mammalian cells known to express p40 include macrophages, dendritic cells, monocytes, Langerhans cells, neutrophils, keratinocytes, plasmacytoid dendritic cells, and microglia (5-10). From cells that express both the p40 and the IL-12-specific p35 subunits, the amount of free p40 secreted is 10-1000 fold higher than the amount of heterodimeric IL-12.

IL-12 and IL-23 are important immunoregulatory molecules. They share overlapping but distinct biological activities, which promote cell-mediated immunity (3, 4). These activities are mediated by the IL-12 and IL-23 receptor complexes that have a common IL-12 receptor beta 1 subunit (IL-12 Rβ1) partnered with the specific IL-12 Rβ2 and IL-23 R subunits, respectively (3, 4, 11-14). Both the monomeric and the dimeric free p40 can also bind IL-12 Rβ1 but not IL-12 Rβ2 or IL-23 R to function as IL-12/IL-23 antagonists. Monomeric p40 binds IL-12 Rβ1 with lower affinity than dimeric p40 and is a less potent antagonist (3, 4, 12-14). Agonistic activities for mouse homodimeric p40 similar to those of heterodimeric IL-12, including the induction of nitric oxide expression and NFκB activation in mouse primary microglia and peritoneal macrophages have been described (3). The molecular mechanism for the agonistic effects of homodimeric p40 has not been determined.

The Quantikine® Mouse IL-12/IL-23 p40 Non Allele-specific Immunoassay is a 4.5 hour solid phase ELISA designed to measure the total amount of mouse p40 subunit from IL-12 p70, IL-23, p40 homodimer, and p40 monomer in cell culture supernates, serum, and plasma. It contains recombinant mouse IL-12/23 p40 homodimer and antibodies raised against the p40 subunit of recombinant mouse IL-12 p70. This immunoassay has been shown to accurately quantitate the recombinant mouse IL-12/IL-23 p40. Results obtained using natural mouse IL-12/IL-23 p40 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-12/IL-23 p40.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-12/IL-23 p40 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-12/IL-23 p40 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-12/IL-23 p40 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-12/IL-23 p40 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, further 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.
- 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 MSDS 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 # MP400	CATALOG # SMP400	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-12/IL-23 p40 Microplate	892897	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-12/IL-23 p40.	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-12/IL-23 p40 Standard	890474	3 vials	9 vials	Recombinant mouse IL-12/IL-23 p40 (dimer) in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard within 8 hours of reconstitution.
Mouse IL-12/IL-23 p40 Control	890455	3 vials	9 vials	Recombinant mouse IL-12/IL-23 p40 (dimer) 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-12/IL-23 p40 Conjugate	892898	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse IL-12/IL-23 p40 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-18	895202	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-4	895435	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.

MP400 contains sufficient materials to run ELISAs on two 96 well plates.
SMP400 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PMP400). 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.
- **Polypropylene** test tubes for dilution of standards and samples.

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 EDTA or 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: *Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.*

SAMPLE PREPARATION

Cell culture supernate samples may contain high levels of IL-12/IL-23 p40. Dilute with Calibrator Diluent RD5-4 when necessary.

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-4.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

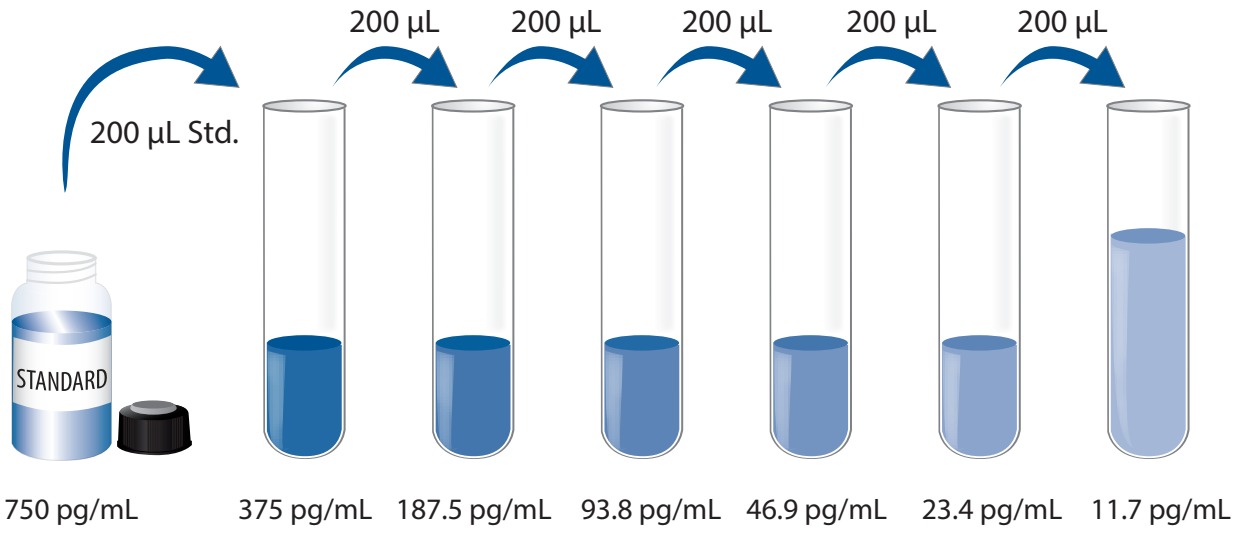
Mouse IL-12/IL-23 p40 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 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-12/IL-23 p40 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-12/IL-23 p40 Standard with Calibrator Diluent RD5-4. Do not substitute other diluents. This reconstitution produces a stock solution of 750 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-4 into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-12/IL-23 p40 Standard (750 pg/mL) serves as the high standard. Calibrator Diluent RD5-4 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, standards, control, and samples as directed by the previous section.
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 RD1-18 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 decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse IL-12/IL-23 p40 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

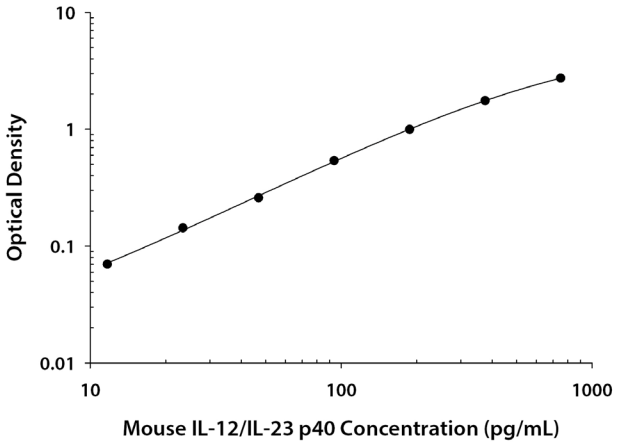
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-12/IL-23 p40 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	0.033 0.037	0.035	—
11.7	0.100 0.110	0.105	0.070
23.4	0.170 0.186	0.178	0.143
46.9	0.292 0.295	0.294	0.259
93.8	0.567 0.581	0.574	0.539
187.5	1.026 1.035	1.031	0.996
375	1.779 1.802	1.791	1.756
750	2.753 2.790	2.772	2.737

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 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	26	21	27
Mean (pg/mL)	33.3	56.4	397	36.5	62.7	405
Standard deviation	2.3	3.0	17.2	3.8	6.5	33.7
CV (%)	6.9	5.3	4.3	10.4	10.4	8.3

RECOVERY

The recovery of mouse IL-12/IL-23 p40 spiked to levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	104	98-108%
Serum* (n=4)	105	86-117%
EDTA plasma* (n=4)	99	81-112%
Heparin plasma* (n=4)	95	80-109%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of mouse IL-12/IL-23 p40 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=7)	Serum* (n=5)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	103	104	109	104
	Range (%)	96-110	100-112	103-117	98-107
1:4	Average % of Expected	107	105	111	105
	Range (%)	95-114	98-118	106-118	95-112
1:8	Average % of Expected	106	108	112	109
	Range (%)	97-113	101-115	101-118	100-114
1:16	Average % of Expected	107	102	105	111
	Range (%)	85-119	88-119	95-116	106-119

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Fourteen assays were evaluated and the minimum detectable dose (MDD) of mouse IL-12/IL-23 p40 ranged from 0.6-2.7 pg/mL. The mean MDD was 1.8 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-12/IL-23 p40 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of mouse IL-12/IL-23 p40 in this assay.

NSA (non-Swiss Albino) Samples	Mean (pg/mL)	Range (pg/mL)
Serum (n=18)	657	365-1083
EDTA plasma (n=9)	632	315-898
Heparin plasma (n=8)	650	394-1176

Note: *Serum samples from two SJL mice were tested for mouse IL-12/IL-23 p40 and averaged 257 pg/mL.*

Cell Culture Supernates:
Bone marrow mast cells (0.2 x 10^6 cells/mL) collected from femurs of SJL mice were cultured in RPMI supplemented with 10% fetal bovine serum and 25 ng/mL of recombinant mouse SCF. 100 ng/mL of recombinant mouse IFN-γ was added on day 12 and 1 µg/mL lipopolysaccharide was added on day 13. On day 15, an aliquot of the cell culture supernate was removed, assayed for mouse IL-12/IL-23 p40, and measured 122 ng/mL.

J774A.1 mouse reticulum cell sarcoma macrophage cells (0.5 x 10^6 cells/mL) were cultured for 1 day in DMEM supplemented with 10% fetal bovine serum containing 20 ng/mL of recombinant mouse IFN-γ and 1 µg/mL lipopolysaccharide. An aliquot of the cell culture supernate was removed, assayed for mouse IL-12/IL-23 p40, and measured 7320 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-12/IL-23 p40.

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-12/IL-23 p40 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-6
IL-7
IL-9
IL-10
IL-10 R
IL-13
JE/MCP-1
KC
Leptin
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant rat:

IL-12 p70

Recombinant human:

IL-12/IL-23 p40 (dimer)
IL-12 p70
IL-23

Recombinant mouse IL-12 p70 cross-reacts approximately 45% in this assay.

Recombinant mouse IL-23 cross-reacts approximately 85% in this assay.

Recombinant mouse IL-12 p40 (monomer) cross-reacts approximately 86% in this assay.

Recombinant rat IL-23 cross-reacts approximately 0.15% in this assay.

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