

# Quantikine<sup>®</sup> ELISA

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

Catalog Number M1240

SM1240

PM1240

For the quantitative determination of mouse Interleukin 12/23 p40 (IL-12/IL-23 p40) 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.**

*\*Polymorphisms exist in the mouse IL-12/IL-23 p40 sequence. The monoclonal capture antibody used in this ELISA kit is allele-specific. It recognizes the IL-12/IL-23 p40 variant with methionine at residue 169 (Accession # P43432) in mouse strains A.SW, BALB/c, C3H, DBA/1, C57BL/6, NOR, and 129/J. The capture antibody does not recognize the M169T IL-12/IL-23 p40 variant present in the AKR, DBA/2, NOD, NZB, NZC, NZO, NZW, and SJL strains (Ymer, S.I. et al. (2002) Genes and Immunity **3**:151). A non allele-specific mouse IL-12/23 p40 ELISA is also available (R&D Systems, Catalog # MP400).*

**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

IL-12, also known as natural killer cell stimulatory factor (NKSF) or cytotoxic lymphocyte maturation factor (CLMF), is a pleiotropic cytokine produced primarily by antigen-presenting cells (monocytes/macrophages, dendritic cells and B lymphocytes). IL-12 has multiple effects on T lymphocytes and natural killer (NK) cells, including the ability to stimulate cytotoxicity, proliferation, cytokine production and Th1 subset development (1, 2).

IL-12 is a disulfide-linked, 70 kDa (p70) heterodimeric glycoprotein composed of a unique 35 kDa (p35) subunit and a common 40 kDa (p40) subunit that is also present in IL-23. Monomers of the p40 and p35 subunits by themselves do not have IL-12 activity, but the homodimer of p40 has been shown to bind the IL-12 receptor and is an IL-12 antagonist (3, 4).

The genes for mouse p40 and p35, found on chromosomes 11 and 3, respectively, are independently regulated (5, 6). The expression of p35 mRNA has been found to be nearly ubiquitous. However, p35 subunits that are not dimerized with the p40 subunits are not secreted and have not been detected in culture supernates of cells expressing only p35 or both p35 and p40 mRNAs (1). In cells expressing both p35 and p40 mRNAs, p40 mRNA is expressed to a higher level and free p40 subunits not associated with p35 subunits are secreted together with heterodimeric IL-12 p70 (7). Most of the free p40 subunits secreted by the various human cell lines examined have been found to exist as monomers (1). In the culture supernates of various activated human monocytes where free p40 is present in vast excess over p70, the levels of p70 measured by bioassays are consistent with those measured using a p70-specific immunoassay, suggesting that p40 monomers are not efficient IL-12 antagonists (1, 8). In the mouse system, p40 homodimers are produced *in vivo* and function as IL-12 antagonists (9).

Polymorphisms exist in the mouse IL-12/IL-23 p40 sequence. The monoclonal capture antibody used in this ELISA kit is allele-specific. It recognizes the IL-12/IL-23 p40 variant with methionine at residue 169 (Accession # P43432) in mouse strains A.SW, BALB/c, C3H, DBA/1, C57BL/6, NOR, and 129/J. The capture antibody does not recognize the M169T IL-12/IL-23 p40 variant present in the AKR, DBA/2, NOD, NZB, NZC, NZO, NZW, and SJL strains (10).

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

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-12/IL-23 p40 variant 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, 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 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.

## MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # M1240	CATALOG # SM1240	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-12/IL-23 p40 Microplate	890472	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 variant.	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. <i>Refer to the vial label for reconstitution volume.</i>	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	890473	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. <i>May turn yellow over time.</i>	
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.

M1240 contains sufficient materials to run ELISAs on two 96 well plates.

SM1240 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM1240). 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.

## 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  $\leq -20$  °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  $\leq -20$  °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.**

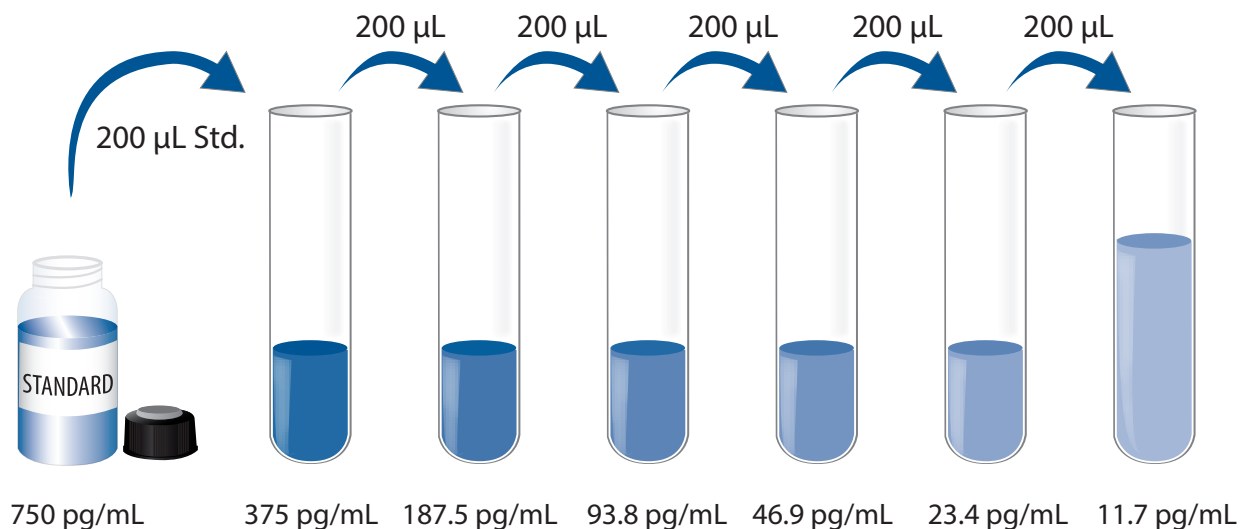
**Mouse IL-12/IL-23 p40 Control** - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

**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  $\mu$ L 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  $\mu$ L of Calibrator Diluent RD5-4 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-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  $\mu\text{L}$  of Assay Diluent RD1-18 to each well.
4. Add 50  $\mu\text{L}$  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  $\mu\text{L}$ ) 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  $\mu\text{L}$  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  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100  $\mu\text{L}$  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

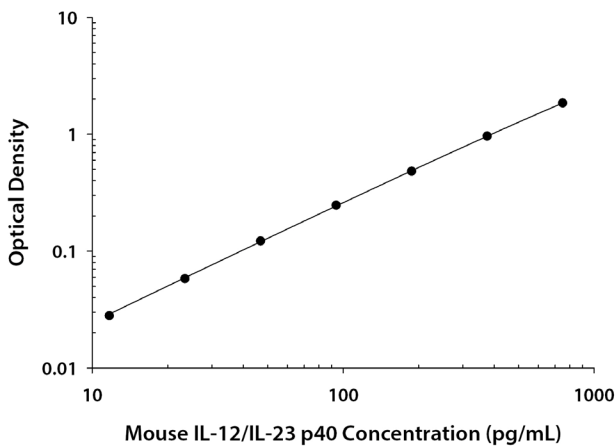
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-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	0.063 0.066	0.064	—
11.7	0.091 0.093	0.092	0.028
23.4	0.123 0.121	0.122	0.058
46.9	0.183 0.188	0.186	0.122
93.8	0.309 0.313	0.311	0.247
187.5	0.545 0.548	0.546	0.482
375	1.028 1.029	1.028	0.964
750	1.895 1.929	1.912	1.848

## 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.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	42.2	161	588	41.9	151	581
Standard deviation	0.81	1.7	9.6	4.1	10.3	24.0
CV (%)	1.9	1.1	1.6	9.8	6.8	4.1

## RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n=11)	100	81-116%
Serum (n=7)	104	85-115%

## LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with various concentrations of mouse IL-12/IL-23 p40 in each matrix were diluted with calibrator diluent and then assayed. Typical data are shown.

Samples	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture supernates	Neat	489	—	—
	1:2	255	244	105%
	1:4	116	122	95%
	1:8	60	61	98%
	1:16	29	30	97%
Serum	Spiked	610	—	—
	1:2	320	305	105%
	1:4	169	152	111%
	1:8	83	76	109%
	1:16	40	38	105%

## SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-12/IL-23 p40 is typically less than 4.0 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** - Samples were evaluated for detectable levels of mouse IL-12/IL-23 p40 in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=40)	149	83	ND-657

ND=Non-detectable

**Cell Culture Supernates** - J774A.1 mouse reticulum cell sarcoma macrophage cells ( $0.5 \times 10^6$  cells/mL) were cultured for 1 day in DMEM supplemented with 10% fetal bovine serum containing 20 ng/mL of recombinant mouse IFN- $\gamma$  and 1.0  $\mu$ g/mL of lipopolysaccharide. An aliquot of the cell culture supernate was removed, assayed for mouse IL-12/IL-23 p40, and measured 999 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	IL-6
G-CSF	IL-7
GM-CSF	IL-9
IFN- $\gamma$	IL-10
IL-1 $\alpha$	IL-10 R
IL-1 $\beta$	IL-13
IL-2	JE/MCP-1
IL-3	KC
IL-4	Leptin
IL-5	LIF

### Recombinant human:

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

Recombinant mouse IL-12 p70 cross-reacts approximately 20% in this assay.

Recombinant mouse IL-23 cross-reacts approximately 53% in this assay.

Recombinant mouse IL-12 p40 (monomer) cross-reacts approximately 52% in this assay.

## REFERENCES

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