Quantikine® ELISA

Mouse IL-12 p70 Immunoassay

Catalog Number M1270
SM1270
PM1270

For the quantitative determination of mouse Interleukin 12 p70 (IL-12 p70) 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 12 (IL-12), also known as NKSF, is a 70-75 kDa heterodimeric glycoprotein that belongs to the IL-12 family of heterodimeric cytokines (1-3). It consists of two disulfide-linked subunits which are 35 kDa (p35) and 40 kDa (p40) in size, and show no meaningful amino acid (aa) sequence identity (1, 4, 5). The mature p35 subunit is 196 aa in length and contains seven cysteines plus one potential N-linked glycosylation site (1-6). Mature mouse p35 shares 63% and 86% aa identity with human and rat p35, respectively (2, 7, 8). Mature mouse p40 is 313 aa in length, with 13 cysteines and five potential N-linked glycosylation sites. Polymorphisms have been reported in the p40 sequence, but these alleles are not recognized by the antibody pair used in this kit (9). Mature mouse p40 shares 72% and 93% aa identity with human and rat p40, respectively (1, 7, 10). While p35 resembles a hematopoietin ligand, p40 strongly resembles the N-terminus of a hematopoietin receptor, exhibiting a WSXWS motif, an immunoglobulin-like domain, and four conserved cysteines (1). This suggests that IL-12 may be a cytokine-receptor analog to the IL-6/soluble IL-6R complex (4, 6). Notably, while p40 may circulate as either a monomer or homodimer, p35 is never found by itself (3). p40 does, however, serve as the larger of two subunits that comprise IL-23 (3, 11). Finally, while IL-12 is classically thought of as a secreted molecule, membrane-bound IL-12 has been reported on both human and mouse cells (12). Cells known to produce IL-12 include macrophages and dendritic cells (13), monocytes (14), Langerhans cells (15), neutrophils (16), keratinocytes (17), plasmacytoid dendritic cells (18), microglia (5), $CD8^+$ dendritic cells (DC) (mouse cells only) (19) and non-germinal center ($CD38^-CD44^+$) B cells (human cells only) (3, 20).

The high affinity receptor for mouse IL-12 is composed of at least two type I transmembrane glycoproteins that resemble members of the cytokine receptor superfamily. The first subunit (Rα1) is 100 kDa in size and binds IL-12 with a Kd=1 nM (21). This receptor serves as the principal binding site for the p40 subunit (4, 5). The second subunit (Rα2) is 130 kDa in size and shows no meaningful aa sequence identity to the Rα1 subunit (5, 21, 22). This receptor appears to be the principal signal transduction component, and is suggested to serve as an attachment point for a disulfide-linked p35-p40 dimer (4, 5, 22). As noted above, mouse p40 will circulate either as a monomer, homodimer, or in a complex bound to either p35, forming IL-12, or to p19, forming IL-23 (3-5, 11). Both the homodimeric p40, and IL-23 can bind to the IL-12R, serving as nonsignalling antagonists (3, 23, 24). Alternatively, the p40 homodimer may also bind to Rα1, activating microglia and macrophages (4, 25).

Functionally, IL-12 has been shown to both enhance cytotoxic activity and induce interferon-gamma (IFN-γ) production in NK cells, T cells and dendritic epidermal T cells (3, 26-28). It has also been reported to induce IFN-γ production in macrophages (29). IL-12, in conjunction with the other IL-12 family members IL-23 and IL-27, is now believed to promote the development of a $CD4^+$ Th1 immune response (4, 5, 30). In response to infection, IL-27 is released initially, promoting a Th0 to Th0/1 transition state. IL-12 follows next, generating Th1 effector cells. With IL-18, IL-12 creates Th1 memory cells out of effector cells, and these cells are later activated by IL-23 (4).

The Quantikine® Mouse IL-12 p70 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-12 p70 in cell culture supernates and mouse serum. It contains recombinant mouse IL-12 expressed in *Sf* 21 insect cells using a baculovirus expression vector and antibodies raised against recombinant mouse IL-12. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IL-12 showed linear curves that were parallel to the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse IL-12 p70.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-12 p70 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any IL-12 p70 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-12 p70 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 p70 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 and 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.

<table>
<tr><th>PART</th><th>PART #</th><th>CATALOG # M1270</th><th>CATALOG # SM1270</th><th>DESCRIPTION</th><th>STORAGE OF OPENED/ RECONSTITUTED MATERIAL</th></tr>
<tr><td>Mouse IL-12 p70 Microplate</td><td>890652</td><td>2 plates</td><td>6 plates</td><td>96 well polystyrene microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-12 p70.</td><td>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.*</td></tr>
<tr><td>Mouse IL-12 p70 Standard</td><td>890654</td><td>1 vial</td><td>3 vials</td><td>Recombinant mouse IL-12 p70 in a buffered protein base with preservatives; lyophilized. Refer to the vial label for reconstitution volume.</td><td rowspan="2">Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze thaw cycles.</td></tr>
<tr><td>Mouse IL-12 p70 Control</td><td>890655</td><td>1 vial</td><td>3 vials</td><td>Recombinant mouse IL-12 p70 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.</td></tr>
<tr><td>Mouse IL-12 p70 Conjugate</td><td>890653</td><td>1 vial</td><td>3 vials</td><td>23 mL/vial of a polyclonal antibody specific for mouse IL-12 p70 conjugated to horseradish peroxidase with preservatives.</td><td rowspan="7">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1-14</td><td>895180</td><td>1 vial</td><td>3 vials</td><td>12 mL/vial of a buffered protein base with preservatives. May contain a precipitate. Mix well before and during use.</td></tr>
<tr><td>Calibrator Diluent RD5Y</td><td>895201</td><td>1 vial</td><td>3 vials</td><td>21 mL/vial of a buffered protein base with preservatives.</td></tr>
<tr><td>Wash Buffer Concentrate</td><td>895003</td><td>2 vials</td><td>6 vials</td><td>21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. May turn yellow over time.</td></tr>
<tr><td>Color Reagent A</td><td>895000</td><td>1 vial</td><td>3 vials</td><td>12 mL/vial of stabilized hydrogen peroxide.</td></tr>
<tr><td>Color Reagent B</td><td>895001</td><td>1 vial</td><td>3 vials</td><td>12 mL/vial of stabilized chromogen (tetramethylbenzidine).</td></tr>
<tr><td>Stop Solution</td><td>895174</td><td>1 vial</td><td>3 vials</td><td>23 mL/vial of diluted hydrochloric acid.</td></tr>
<tr><td>Plate Sealers</td><td>N/A</td><td>8 strips</td><td>24 strips</td><td colspan="2">Adhesive strips.</td></tr>
</table>

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

M1270 contains sufficient materials to run ELISAs on two 96 well plates.
SM1270 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM1270). 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 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 ≤ -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.

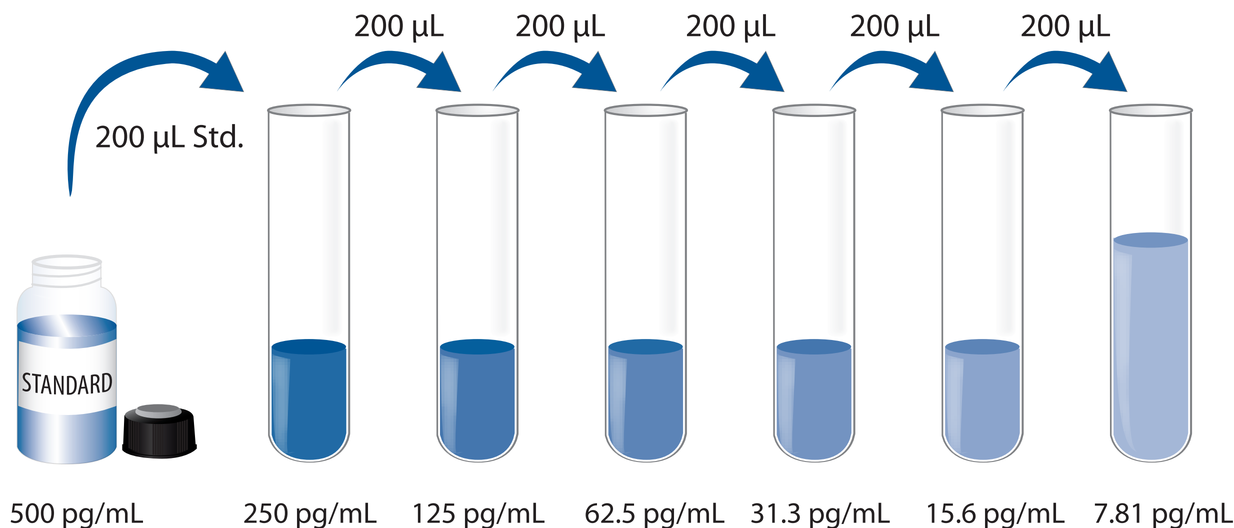
Mouse IL-12 p70 Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 into 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. 100 μL of the resultant mixture is required per well.

Mouse IL-12 p70 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-12 p70 Standard with Calibrator Diluent RD5Y. 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.

Use polypropylene tubes. Pipette 200 μL of Calibrator Diluent RD5Y 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 p70 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5Y 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 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. Mix well before and during use.*
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. A plate layout is provided to record standards and samples assayed.
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 p70 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

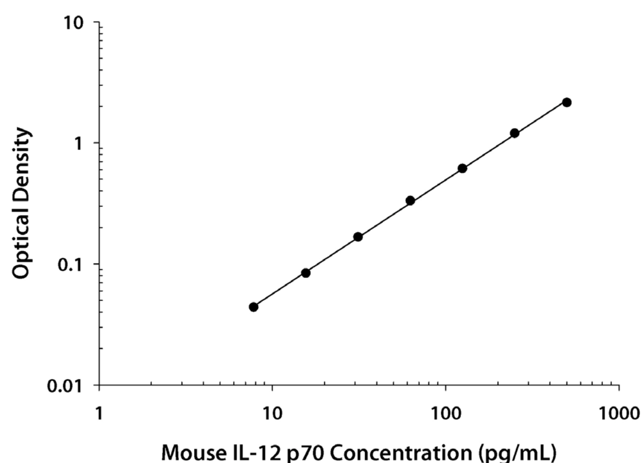
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-12 p70 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	0.012 0.013	0.012	—
7.81	0.053 0.058	0.056	0.044
15.6	0.096 0.097	0.096	0.084
31.3	0.177 0.181	0.179	0.167
62.5	0.343 0.347	0.345	0.333
125	0.616 0.636	0.626	0.614
250	1.193 1.229	1.211	1.199
500	2.157 2.172	2.164	2.152

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	36	44	36
Mean (pg/mL)	28	68	193	20	56	230
Standard deviation	2.5	6.5	19.4	2.3	6.2	20.0
CV (%)	8.9	9.6	10.1	11.5	11.1	8.7

RECOVERY

The recovery of mouse IL-12 p70 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=7)	101	95-106%
Serum (n=7)	97	88-104%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of mouse IL-12 p70 in each matrix were diluted with calibrator diluent and then assayed.

		Cell culture supernates (n=3)	Serum (n=3)
1:2	Average % of Expected	105	104
	Range (%)	101-108	104-105
1:4	Average % of Expected	105	103
	Range (%)	102-110	100-106
1:8	Average % of Expected	108	109
	Range (%)	106-110	106-113
1:16	Average % of Expected	105	107
	Range (%)	104-108	105-110

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SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-12 p70 is typically less than 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 *Sf* 21-expressed recombinant mouse IL-12 p70 produced at R&D Systems®.

SAMPLE VALUES

Serum - Forty mouse serum samples were evaluated for detectable levels of IL-12 p70 in this assay. Thirty-nine samples read below the lowest standard, 7.81 pg/mL. One sample read 21 pg/mL.

Cell Culture Supernates:
Bone marrow mast cells collected from femurs of Balb/c mice were cultured (1×10^5 cells/mL) in RPMI supplemented with 10% fetal bovine serum and 25 ng/mL recombinant mouse SCF. Recombinant mouse IFN-γ (100 ng/mL) was added on day 12 and LPS (1.0 μg/mL) was added on day 13. At day 15 the cell culture supernate was tested for mouse IL-12 p70 and read 101 pg/mL.

Mouse spleen cells (1×10^6 cells/mL) were cultured for 2-4 days in RPMI supplemented with 10% fetal bovine serum, 100 ng/mL mouse IFN-γ, and 1.0 μg/mL LPS. The cell culture supernate was assayed for mouse IL-12 p70 and measured 59 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-12 p70.

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 p70 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-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12 p35
IL-12/IL-23 p40 monomer
IL-12/IL-23 p40 dimer
IL-13
IL-23
JE/MCP-1
KC
Leptin
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant human:

G-CSF
IL-6
IL-6 R
IL-12 p35
IL-12/IL-23 p40 monomer
IL-12 p70
IL-23

Recombinant rat IL-12 cross-reacts approximately 48% in this assay.

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PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

NOTES

NOTES

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