Quantikine® ELISA

Mouse CXCL10/IP-10/CRG-2 Immunoassay

Catalog Number MCX100
SMCX100
PMCX100

For the quantitative determination of mouse interferon gamma Inducible Protein 10 (IP-10) 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.

TABLE OF CONTENTS

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INTRODUCTION

Interferon-gamma inducible protein (IP-10) is a non-ELR CXC chemokine belonging to the chemokine superfamily and has been designated CXCL10 (1, 2). Mouse IP-10, also known as cytokine response gene-2 (CRG-2), was originally identified in monocytes as an interferon- or lipopolysaccharide-inducible gene (3, 4). Mouse IP-10 cDNA encodes a 98 amino acid (aa) residue precursor protein with a putative 21 aa signal peptide and a 77 aa secreted mature protein with no potential N-linked glycosylation sites. As a result of proteolytic processing by CD26 and other proteases, both N- and C-terminally truncated IP-10 isoforms exist (5-7). Mature mouse IP-10 shares 70% and 75% aa sequence identity with human IP-10 and rat IP-10, respectively (7-9). By similarity to other chemokines, IP-10 has two intrachain disulfide bonds. In solution, recombinant human IP-10 has been shown to exist as noncovalent dimers at millimolar concentrations (10). IP-10 is expressed in a number of cell types including hepatocytes (11), monocyte-derived dendritic cells (12), thymic medullary cells (13), $CD4^+$ Th1 T cells (14), testicular seminiferous tubular and Sertoli cells (15), specialized vascular smooth muscle cells (16), neutrophils (17), fibroblasts (18), γδ T cells (19), astrocytes and endothelial cells (20), thyrocytes (21), bronchial epithelial cells (22), keratinocytes (23), macrophages (24), and monocytes (25). The expression is highly upregulated by interferon-γ, a typical Th1 cytokine (1-4).

IP-10 is a chemoattactant that signals via binding and activation of the seven transmembrane G-protein-coupled receptor CXCR3, a CXC chemokine receptor that is expressed in T cells (especially activated Th1 cells), B cells, NK cells, dendritic cells, $CD34^+$ hematopoietic progenitor cells, subsets of thymocytes and endothelial cells (12, 13, 25-31). The wide range of cells expressing CXCR3 suggest that IP-10 may play a role in T cell lymphopoiesis, stem cell differentiation and the development of allergic and type 1 inflammatory responses (13, 28, 30, 31). Besides its chemotactic properties, IP-10 was reported to be anti-angiogenic and can inhibit endothelial cell proliferation in a cell-cycle-dependent manner (32, 33). IP-10 has also been found to have defensin-like anti-microbial activity (34).

In addition to CXCR3, IP-10 binds CCR3, a CC chemokine receptor preferentially expressed on Th2 cells (10, 35). However, in this case, IP-10 acts as an antagonist that inhibits CCR3-mediated chemotaxis. These opposing effects of IP-10 on CXCR3- vs CCR3-bearing cells can enhance the polarization of Th1 cell recruitment to sites of inflammation. Like many chemokines, IP-10 also binds to cell surface glycosaminoglycans (GAG) (27, 36). The function of the GAG-chemokine interactions remains to be defined. In cells that lack CXCR3 and GAG, yet another functional IP-10-specific receptor has been postulated (37).

The Quantikine Mouse CXCL10/IP-10/CRG-2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse IP-10 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IP-10 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse IP-10 showed linear 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 naturally occurring mouse IP-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse IP-10 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse IP-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IP-10 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 IP-10 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 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.

PART	PART #	CATALOG # MCX100	CATALOG # SMCX100	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IP-10 Microplate	892349	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with polyclonal antibody specific for mouse IP-10.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Mouse IP-10 Standard	892351	2 vials	9 vials	Recombinant mouse IP-10 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.
Mouse IP-10 Control	892352	2 vials	9 vials	Recombinant mouse IP-10 in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Mouse IP-10 Conjugate	892350	1 vial	6 vials	12 mL/vial of a polyclonal antibody against mouse IP-10 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1N	895488	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-17	895512	1 vial	3 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	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	4 strips	24 strips	Adhesive strips.	

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

MCX100 contains sufficient materials to run an ELISA on one 96 well plate.
SMCX100 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMCX100). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.

PRECAUTIONS

Assay Diluent RD1N contains sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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. Please 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 and citrate plasma have not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

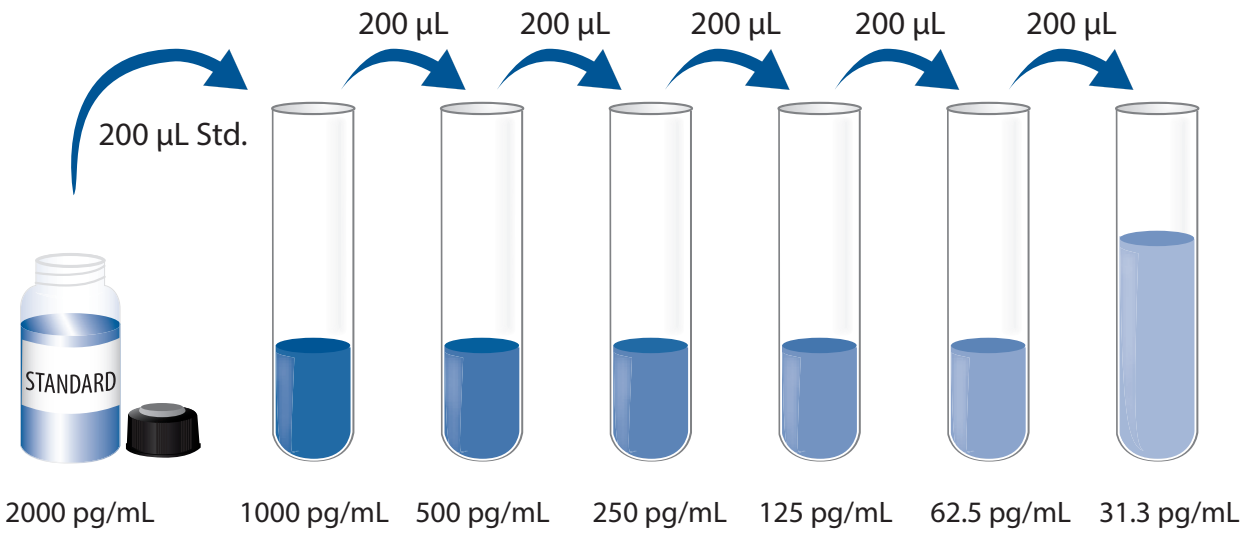
Mouse IP-10 Control - Reconstitute the Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

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

Mouse IP-10 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IP-10 Standard with Calibrator Diluent RD5-17. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 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 RD5-17 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 IP-10 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD5-17 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, standard dilutions, 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 RD1N to each well.
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 IP-10 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

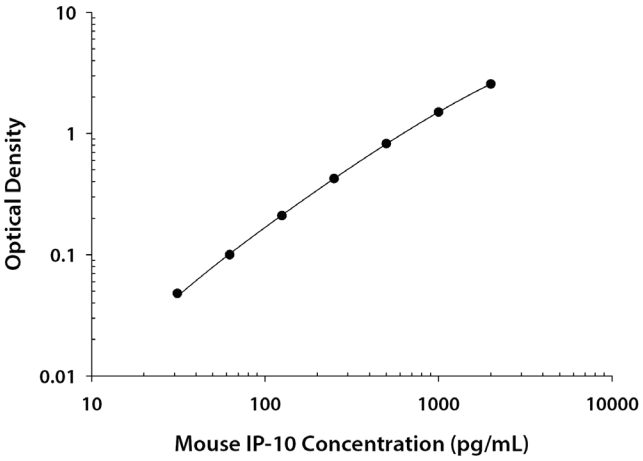
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 IP-10 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	0.025 0.026	0.026	—
31.3	0.074 0.075	0.074	0.048
62.5	0.124 0.129	0.126	0.100
125	0.226 0.247	0.236	0.210
250	0.443 0.458	0.450	0.424
500	0.843 0.858	0.850	0.824
1000	1.515 1.541	1.528	1.502
2000	2.535 2.638	2.586	2.560

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	106	217	836	107	250	874
Standard deviation	4.2	10	50	11	20	67
CV (%)	4.0	4.6	6.0	10.3	8.0	7.7

RECOVERY

The recovery of mouse IP-10 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	97	88-104%
Serum (n=5)	97	81-118%
Heparin plasma (n=6)	109	93-118%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of mouse IP-10 in each matrix were diluted with Calibrator Diluent and then assayed.

		Cell culture supernates (n=4)	Serum (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	95	104	96
	Range (%)	89-107	101-106	89-104
1:4	Average % of Expected	100	111	94
	Range (%)	93-111	110-113	84-107
1:8	Average % of Expected	98	112	94
	Range (%)	91-111	107-117	81-105
1:16	Average % of Expected	93	109	99
	Range (%)	81-105	103-112	82-102

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse IP-10 produced at R&D Systems.

SENSITIVITY

Thirty-eight assays were evaluated and the minimum detectable dose (MDD) of mouse IP-10 ranged from 1.2-4.2 pg/mL. The mean MDD was 2.2 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.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for the presence of mouse IP-10 in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum	78	45	ND-226
Heparin plasma	49	49	ND-106

ND=Non-detectable

Cell Culture Supernates:
Bone marrow mast cells collected from femurs of Balb/c mice were cultured (1 x 10^5 cells/mL) in RPMI containing 10% fetal bovine serum and 25 ng/mL recombinant mouse (rm) SCF. 100 ng/mL of rmIFN-γ was added on day 12 and 1 µg/mL of LPS was added on day 13. An aliquot of the cell culture supernate was removed on day 15, assayed for mouse IP-10, and measured 1294 pg/mL.

RAW 264.7 mouse monocyte/macrophage cells (1 x 10^6 cells/mL) were cultured in DMEM containing 10% fetal bovine serum and grown to 85% confluency. Fresh medium with rmIFN-γ (100 ng/mL) was added and incubated for one day. 2 µg/mL of LPS was added on day two and incubated for 24 hours. An aliquot of the cell culture supernate was removed on day 3, assayed for mouse IP-10, and measured 1484 pg/mL.

J774A.1 mouse reticulum cell sarcoma macrophage cells (1 x 10^6 cells/mL) were cultured for 3 days in DMEM containing 10% fetal bovine serum and stimulated with 2.5 ng/mL of LPS. An aliquot of the cell culture supernate was removed, assayed for mouse IP-10, and measured 1571 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IP-10.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the same factors at 50 ng/mL in a mid-range mouse IP-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:
BLC/BCA-1
GCP-2
I-TAC
KC
MIG
SDF-1α

Recombinant human:
BLC/BCA-1
IL-8
IP-10
MIG

Recombinant porcine:
IL-8

Note: *Normal rat serum and cell culture supernate samples were also evaluated and measured less than the lowest standard, 31.3 pg/mL.*

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