Quantikine® ELISA

Mouse CXCL9/MIG Immunoassay

Catalog Number MCX900

For the quantitative determination of mouse Monokine Induced by IFN-γ (MIG) concentrations in tissue homogenates, cell culture supernates, serum, and plasma.

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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614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

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19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
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24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
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INTRODUCTION

Mouse Monokine Induced by interferon-gamma (MIG; CXCL9) is an 11 kDa non-ELR (Glu-Leu-Arg) containing chemoattractant that belongs to the alpha-, or C-X-C family of chemokines (1, 2). MIG is synthesized as a 126 amino acid (aa) precursor that contains a 21 aa signal sequence plus a 105 aa mature segment (3). Unlike human MIG, mouse MIG is glycosylated (3, 4). Intracellular and extracellular proteolytic processing likely occurs, creating both N- and C-terminally truncated forms. In humans, removal of Thr and Pro by CD26 at the N-terminus results in a marginal reduction in activity (5). At the C-terminus, intracellular proteolysis creates multiple short forms, which in the human are 78, 81 and 91 aa in length (4, 6). Short forms have shown decreased receptor binding, possibly through disruption of the MIG C-terminal α-helix (6). There is 72% aa sequence identity in the mature segment between mouse to human MIG (3, 7). In the mouse, mature MIG shows 34% and 36% aa sequence identity to mature I-TAC/CXCL11 and IP-10/CXCL10, respectively (8). Cells known to express MIG include thymic medullary epithelium (9), macrophages (10, 11), thyroid epithelium (11, 12), basal layer keratinocytes (13), $CD8\alpha^+$ and $CD8\alpha^-$ dendritic cells (14), astrocytes (15), capillary and venous endothelium (15, 16), hepatocytes (17), and salivary ductal epithelium (18).

The receptor for MIG is the 41 kDa, 7-transmembrane G-protein coupled receptor termed CXCR3 (19, 20). The receptor is found on a number of hematopoietic cell types including $CD34^+$ stem cells (21), monocytes (16), resting memory and activated $CD4^+$ and $CD8^+$ T cells (particularly Th1 cells) (14), $CD4^+$ thymocytes, $CD8^+$ thymocytes, and γδ T cells (9).

MIG, by definition, is IFN-γ inducible and is involved in Th1-type inflammation. It has a number of functions, the principal being chemoattraction. Cells known to respond to MIG include activated B cells (14), $CD34^+$ stem cells (21), monocytes (16), $CD56^+$ NK cells (9), $CD8^+$ memory T cells (9, 22), and $CD4^+$ Th1 T cells (23). It also promotes Th1 responses by serving as an antagonist to IL-4/Th2 responses. This occurs by blocking Th2-related chemokine (eotaxin) binding to CCR3, thus limiting Th2 infiltration (24). MIG also demonstrates direct anti-microbial activity, apparently through its extended C-terminus (25). During hematopoiesis, MIG is involved in the mobilization and migration of $CD34^+$ stem cells (21), and the suppression of colony formation of both erythroid (BFU-E) and myeloid (CFU-GM) lineage cell types (25, 26).

The Quantikine® Mouse CXCL9/MIG Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse MIG in tissue homogenates, cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse MIG and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse MIG 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 natural mouse MIG.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for mouse MIG has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any MIG present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse MIG 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 MIG 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 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.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse MIG Microplate	892230	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse MIG.	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 MIG Standard	892232	2 vials of recombinant mouse MIG 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 after use.
Mouse MIG Control	892233	2 vials of recombinant mouse MIG in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse MIG Conjugate	892231	12 mL of a polyclonal antibody specific for mouse MIG conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-63	895352	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-4	895435	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

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 and samples.

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. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Tissue Homogenates - Spleen and liver tissue from two mice were rinsed with PBS to remove excess blood, homogenized in 20 mL of PBS, and stored overnight at ≤ -20 °C. After two freeze-thaw cycles, centrifuge the homogenates for 5 minutes at 5000 x g. Assay the supernate 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 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: *Heparin 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.

SAMPLE PREPARATION

Use polypropylene tubes.

Serum and plasma samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-4.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

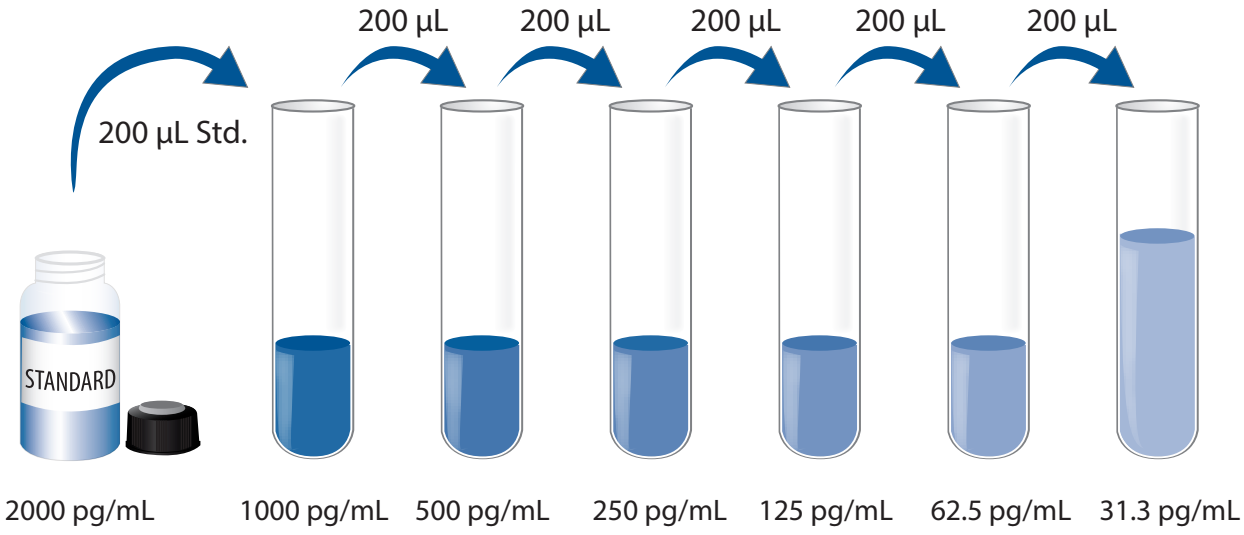
Mouse MIG Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of 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 MIG Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse MIG Standard with Calibrator Diluent RD5-4. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the stock solution 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 dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse MIG Standard (2000 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 standards, control, and samples 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 RD1-63 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 MIG 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

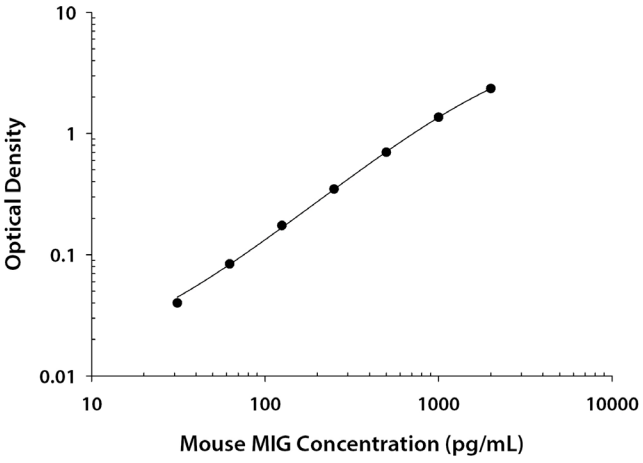
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 MIG 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	0.052 0.055	0.054	—
31.3	0.093 0.094	0.094	0.040
62.5	0.136 0.139	0.138	0.084
125	0.225 0.230	0.228	0.174
250	0.398 0.407	0.402	0.348
500	0.748 0.760	0.754	0.700
1000	1.382 1.454	1.418	1.364
2000	2.348 2.450	2.399	2.345

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-three separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of kit components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	23	23	23
Mean (pg/mL)	77	216	815	66	190	741
Standard deviation	6.2	14.8	68	5.5	14.1	39.5
CV (%)	8.1	6.9	8.3	8.3	7.4	5.3

RECOVERY

The recovery of mouse MIG spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	95	81-116%
Tissue homogenates (n=2)	104	88-118%
Serum* (n=9)	93	85-112%
EDTA plasma* (n=9)	94	81-107%

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

LINEARITY

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

		Cell culture supernates (n=4)	Tissue Homogenates (n=2)	Serum* (n=6)	EDTA plasma* (n=6)
1:2	Average % of Expected	92	108	94	100
	Range (%)	89-96	98-118	91-98	94-107
1:4	Average % of Expected	92	102	94	101
	Range (%)	89-99	98-107	88-102	94-109
1:8	Average % of Expected	92	89	92	102
	Range (%)	86-94	———	84-97	97-112
1:16	Average % of Expected	95	———	92	101
	Range (%)	93-97	———	81-100	92-110

*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 MIG ranged from 0.98-7.8 pg/mL. The mean MDD was 3.0 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 *E. coli*-expressed recombinant mouse MIG produced at R&D Systems®.

SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=20)	148	85	ND-592
EDTA plasma (n=20)	142	65	ND-350

ND=Non-detectable

Cell Culture Supernates - 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 500 ng/mL recombinant mouse IFN-γ. An aliquot of the cell culture supernate was removed, assayed for mouse MIG, and measured 1154 pg/mL.

Tissue Homogenates - The tissue homogenates from spleen and liver tissue were assayed for mouse MIG and measured 158 pg/mL and 322 pg/mL, respectively.

SPECIFICITY

This assay recognizes natural and recombinant mouse MIG.

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 MIG control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

BLC/BCA-1
IP-10/CRG-2
I-TAC
LIX
SDF-1α

Recombinant human:

BLC/BCA-1
GCP-2
IL-8
IP-10
I-TAC
MIG
NAP-2

Recombinant rat:

LIX

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