Quantikine™ ELISA

Human MIF Immunoassay

Catalog Number DMF00B
SMF00B
PDMF00B

For the quantitative determination of human Macrophage Migration Inhibitory Factor (MIF) concentrations in cell culture supernates, serum, plasma, urine, and human milk.

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

One of the first cytokines described, MIF (macrophage migration inhibitory factor) was originally shown to inhibit random macrophage migration, but was later found to be chemotactic (1-4). With the discovery that MIF is a non-canonical ligand of chemokine receptors, MIF is now proposed to belong to a group of chemokine-like function (CLF) proteins, or "microchemokines" (3, 4). It is an important mediator of the innate immune response with potential roles in the pathophysiology of inflammatory, autoimmune, and neoplastic disorders (3-5). The human MIF gene encodes a 115 amino acid, 12.5 kDa secreted protein (6). Crystallography studies suggest that MIF exists as a homotrimer (7), but dimers or monomers are also possible (8, 9). MIF shares minimal homology with other known cytokines. It exhibits intrinsic tautomerase and oxidoreductase activity, but the physiological importance of MIF as an enzyme remains unclear (3, 4, 10, 11).

MIF is expressed by cells of the immune system, including monocytes/macrophages, T and B cells, neutrophils, and atherosclerotic foam cells (3, 4, 12). It also exhibits non-immune expression, such as in endothelium, smooth muscle, cardiomyocytes, tumor neovasculature and endocrine tissues including pancreatic β cells, adrenal and pituitary glands (3, 4, 13-15). MIF can be constitutively expressed and maintained in intracellular pools prior to its secretion (16). Some MIF activities require binding to CD74, a cell surface form of the MHC class II invariant chain, using CD44 as a signaling coreceptor (17, 18). For others, MIF binds the chemokine receptors, CXCR4 and/or CXCR7, giving MIF chemokine-like activity (5). These receptors can also form a complex with CD74 (5, 19). MIF also exhibits receptor-independent uptake and signaling, binding intracellular Jab1 and relieving Jab1-mediated G1 cell cycle arrest (20, 21).

MIF mediates both acute and chronic inflammatory responses, and is released into circulation following endotoxin treatment or by pro-inflammatory cytokines including TNF-α and IFN-β (3, 15, 16). MIF has potent paracrine and autocrine stimulatory effects on cell growth and survival and enhances the production of inflammatory cytokines (16, 22-24). MIF is also known for its inhibitory effects on immunosuppressive glucocorticoids (13, 22). Neutralizing MIF antibodies or knockout of the MIF gene inhibits mouse models of endotoxemia or septic shock, while treatment with the recombinant protein exacerbates toxicity (15, 25, 26). MIF induces upregulation of toll-like receptor 4 (TLR4), which mediates the pro-inflammatory role of MIF in response to endotoxin (27, 28). In humans, MIF is elevated in the plasma of patients with sepsis or septic shock and in the lungs of patients with acute respiratory distress syndrome (ARDS) (25, 29-32). Elevated levels of MIF are also found in the synovial fluid of rheumatoid arthritis (RA) patients, and blockade of MIF suppresses inflammation in mouse models of RA (33, 34).

Elevated plasma levels of MIF are associated with atherosclerotic lesions, leukocyte recruitment and CXCR2-mediated chronic inflammatory responses following arterial wall injury (3, 4, 35, 36). MIF is also thought to be a factor in obesity- and type 2 diabetes-related chronic inflammation (14, 37). MIF expression is increased in several forms of cancer (4, 38, 39). In animal models, tumor growth is inhibited by blocking MIF activity and enhanced by transgenic over-expression, and MIF can inhibit p53 tumor suppressor activity, enhance the migratory and/or invasive capabilities of tumor cells, and promote VEGF expression and angiogenesis (3, 4, 39). In humans, MIF expression has been correlated with an increase in vessel density and enhanced risk of tumor recurrence (39). MIF is thought to mediate the increased risk of cancer seen in chronic inflammatory conditions (40).

The Quantikine™ Human MIF Immunoassay is a 4.5 hour solid phase ELISA designed to measure human MIF in cell culture supernates, serum, plasma, urine, and human milk. It contains *E. coli*-expressed recombinant human MIF and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human MIF 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 human MIF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MIF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MIF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MIF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of MIF bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

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

PART	PART #	CATALOG # DMF00B	CATALOG # SMF00B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human MIF Microplate	891019	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MIF.	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.*
Human MIF Conjugate	891020	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human MIF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human MIF Standard	891021	1 vial	6 vials	Recombinant human MIF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-53	895318	1 vial	3 vials	21 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-20 Concentrate	895346	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
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	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DMF00B contains sufficient materials to run an ELISA on one 96 well plate.
SMF00B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDMF00B). 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
- 100 mL and 500 mL graduated cylinders
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- Test tubes for dilution of standards and samples
- Human MIF Controls (optional; R&D Systems®, Catalog # QC122)

PRECAUTIONS

MIF is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 SDS 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 ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Platelet-Poor Plasma - Collect plasma on ice using EDTA or heparin as an anticoagulant. Centrifuge at 2-8 °C for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the separated plasma at 10,000 x g for 10 minutes at 2-8 °C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Note: *Do not use hemolyzed samples.*
Citrate plasma has not been validated for use in this assay.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -70 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and platelet-poor plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 50 µL of sample + 450 µL of Calibrator Diluent RD5-20 (diluted 1:5)*.

Urine samples require a 5-fold dilution. A suggested 5-fold dilution is 100 µL of sample + 400 µL of Calibrator Diluent RD5-20 (diluted 1:5).

Human milk samples require a 100-fold dilution. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5-20 (diluted 1:5).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of MIF are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

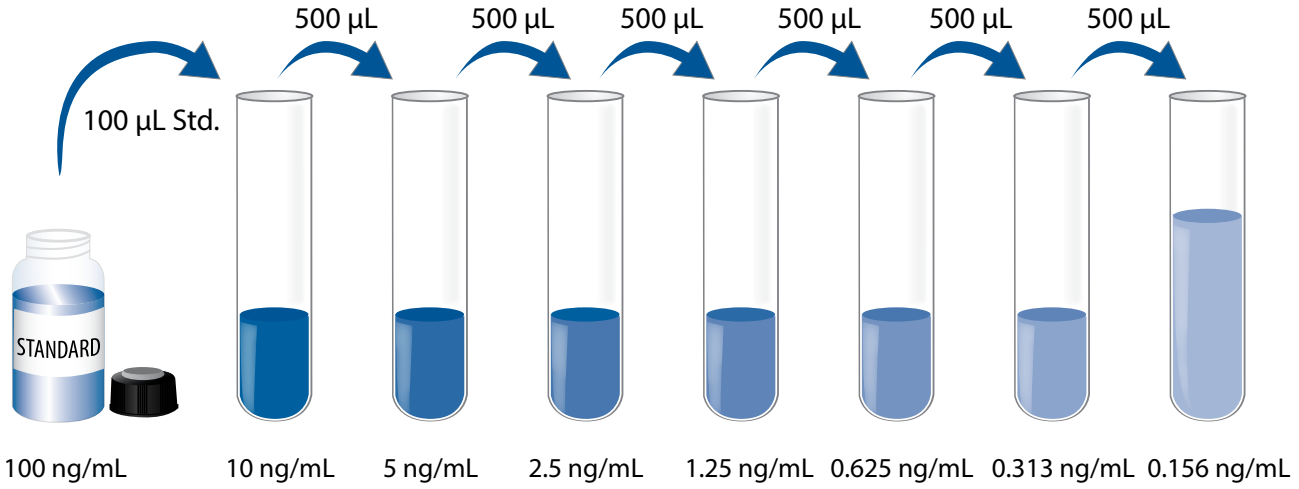
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-20 (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5-20 Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5-20 (diluted 1:5).

Human MIF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MIF Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 ng/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-20 (diluted 1:5) into the 10 ng/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10 ng/mL standard serves as the high standard. Calibrator Diluent RD5-20 (diluted 1:5) serves as the zero standard (0 ng/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples be assayed in duplicate.

Note: *High concentrations of MIF are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1-53 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. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 µL of Human MIF 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

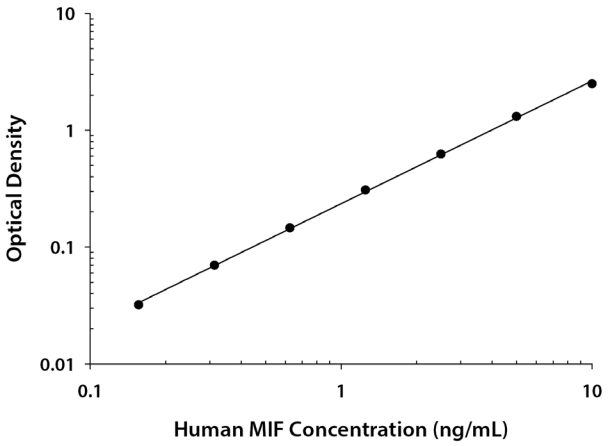
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 human MIF concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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.



(ng/mL)	O.D.	Average	Corrected
0	0.041 0.041	0.041	—
0.156	0.073 0.073	0.073	0.032
0.313	0.109 0.113	0.111	0.070
0.625	0.179 0.194	0.187	0.146
1.25	0.343 0.354	0.349	0.308
2.5	0.656 0.673	0.665	0.624
5	1.311 1.392	1.352	1.311
10	2.530 2.551	2.541	2.500

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 forty 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	40	40	40
Mean (ng/mL)	1.16	3.56	7.27	1.03	3.20	6.46
Standard deviation	0.07	0.18	0.33	0.10	0.27	0.61
CV (%)	6.0	5.1	4.5	9.7	8.4	9.4

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	95	86-104%
Serum* (n=4)	99	94-104%
Platelet-poor EDTA plasma* (n=5)	95	80-108%
Platelet-poor heparin plasma* (n=5)	96	86-108%

*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 human MIF were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=4)	Platelet-poor		Urine* (n=4)	Human milk* (n=4)
				EDTA plasma* (n=5)	Heparin plasma* (n=5)		
1:2	Average % of Expected	108	103	106	105	105	102
	Range (%)	99-118	100-104	94-113	99-114	101-108	96-108
1:4	Average % of Expected	108	102	104	107	110	104
	Range (%)	104-113	94-105	98-110	101-111	104-117	95-114
1:8	Average % of Expected	111	102	103	106	110	104
	Range (%)	108-114	99-104	93-113	100-113	102-112	98-112
1:16	Average % of Expected	105	100	106	103	102	106
	Range (%)	103-110	95-102	100-114	101-106	94-111	97-113

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

SENSITIVITY

Forty-three assays were evaluated and the minimum detectable dose (MDD) of human MIF ranged from 0.005-0.068 ng/mL. The mean MDD was 0.016 ng/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 human MIF produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human MIF in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=36)	22.3	15.3-52.3	6.1
Platelet-poor EDTA plasma (n=20)	26.8	16.5-72.6	12.3
Platelet-poor heparin plasma (n=20)	16	11.2-25.4	3.6
Urine (n=16)	7.1	2.5-16.5	3.8
Human milk (n=8)	796	468-1058	188

Current research is inconclusive on the relationship of human MIF and platelets. Data obtained at R&D Systems indicate that sample values obtained from platelet-poor plasma are more consistent with serum values; therefore, platelet-poor plasma is recommended.

Cell Culture Supernates:
Human peripheral blood lymphocytes were cultured in DMEM supplemented with 5% fetal bovine serum, 5 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human MIF.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	7.74	9.84
Stimulated	12.2	14.9

SAMPLE VALUES *CONTINUED*

Human monocytes were separated in Ficoll®-Hypaque™ and then cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated with 1 µg/mL LPS for 30 hours. An aliquot of the cell culture supernate was removed, assayed for human MIF, and measured 3.47 ng/mL.

Human dendritic cells were differentiated from human monocytes by culture in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 100 ng/mL recombinant human GM-CSF, and 10 ng/mL recombinant human IL-4. Cells were stimulated with 10 ng/mL of recominant human TNF-α on day 6. An aliquot of the cell culture supernate was removed on day 10, assayed for human MIF, and measured 9.85 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human MIF.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human MIF standard were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

BLC/BCA-1
ENA-78
GCP-2
GROα
GROβ
GROγ
IL-8
IP-10
I-TAC
MIG
NAP-2
SDF-1α
SDF-1β
Thioredoxin

Recombinant mouse:

BLC/BCA-1
CRG-2/IP-10
GCP-2
KC
MIG
MIF
SDF-1α

Recombinant porcine:

IL-8

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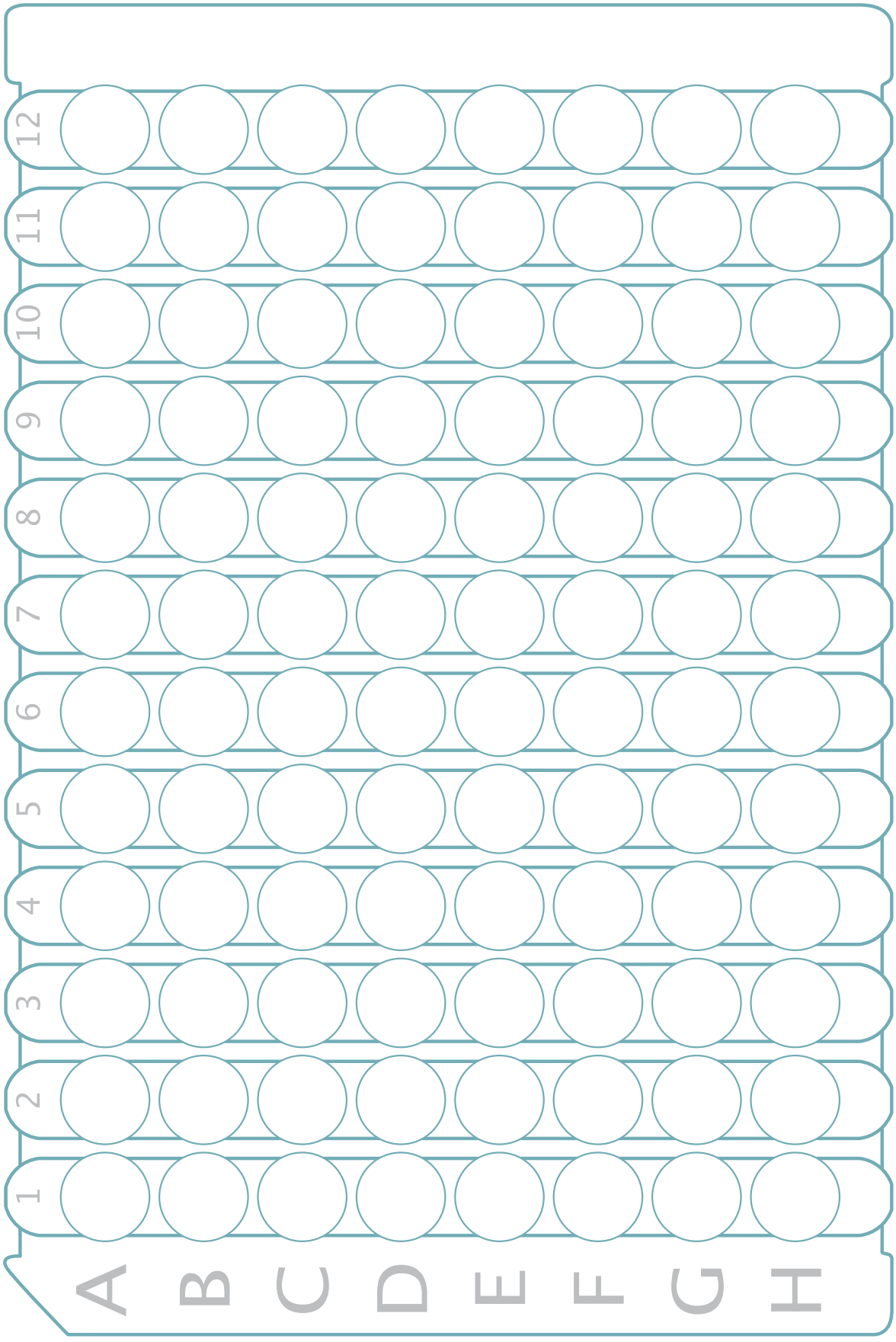
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

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