

# Quantikine<sup>®</sup>

## Human MIF Immunoassay

Catalog Number DMF00  
SMF00  
PDMF00

**For the quantitative determination of human Macrophage Migration Inhibitory Factor (MIF) concentrations in 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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## INTRODUCTION

One of the first cytokines described, MIF (macrophage migration inhibitory factor) was originally identified in studies of delayed hypersensitivity reactions where it was shown to inhibit macrophage migration (1, 2). It is an important mediator of the innate immune response with potential roles in the pathophysiology of inflammatory, autoimmune, and neoplastic disorders (3, 4). The human MIF gene encodes a 115 amino acid, 12.5 kDa secreted protein (5). Crystallographic studies suggest that MIF exists as a homotrimer (6), although some reports show that it may exist as a dimer or monomer as well (7, 8). Although MIF exhibits no homology with other known cytokines, it shares structural homology with several bacterial enzymes (9, 10). MIF exhibits intrinsic catalytic activity and has been shown to act as a tautomerase and an oxidoreductase, although its physiological importance as an enzyme remains unclear (11 - 17).

MIF is expressed by several cells of the immune system including T cells, monocytes/macrophages, B cells, neutrophils, eosinophils, mast cells, and basophils (3, 18). It is also expressed in several non-immune cell types and tissues including the epithelial linings of the lung, skin, and gastrointestinal tract, and endocrine tissues including the adrenal and pituitary glands (3, 18). Unlike many cytokines that are expressed upon cell activation, MIF can be constitutively expressed and maintained in intracellular pools prior to its secretion (19 - 23). Recently, some MIF activities have been shown to require binding to CD74, a cell surface form of the MHC class II invariant chain (24). However, CD74 contains no intracellular domain consistent with signaling capabilities and whether it is the sole receptor mediating MIF activity is unknown. MIF could potentially be taken up by cells and signal in a receptor-independent manner (25). Yeast two-hybrid studies reveal that MIF binds intracellular Jab1, and in fibroblasts this interaction suppresses Jab1 stimulation of JNK, AP1, and relieves Jab1-mediated G1 cell cycle arrest (26).

MIF is well known as a mediator of acute and chronic inflammatory responses and is released into circulation following endotoxin treatment or by pro-inflammatory cytokines including TNF- $\alpha$  and IFN- $\gamma$  (3, 19 - 21). Working in a paracrine or autocrine manner, MIF has potent stimulatory effects on cell growth and survival and enhances the production of an array of inflammatory cytokines (20, 27 - 29). MIF is also known for its inhibitory effects on immunosuppressive glucocorticoids (27). 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 (19, 30, 31). Part of the mechanism underlying MIF-induced pro-inflammatory activities is an upregulation of toll-like receptor 4 (TLR4) (32). In humans, MIF levels are known to increase in patients with sepsis or septic shock (30, 33, 34). MIF is also elevated in the lungs of patients with acute respiratory distress syndrome (ARDS), a hyper-inflammatory response that can accompany sepsis or lung injury (33, 35, 36). Blockade of MIF activity suppresses inflammation associated with mouse models of rheumatoid arthritis (RA), and elevated levels of MIF are found in the synovial fluid of RA patients (37, 38). In addition, a polymorphism that results in decreased MIF promoter activity correlates with low disease severity in RA patients (39). Eosinophils are a potential source for MIF found elevated in bronchoalveolar lavage fluid from asthmatic patients (22). In addition, elevated levels are associated with atherosclerotic lesions, suggesting that MIF may function in chronic inflammatory responses that accompany arterial wall injury (40).

MIF expression is increased in several forms of cancer (4, 41). In cancer models, tumor growth is inhibited by blocking MIF activity and enhanced by transgenic over-expression (42 - 46). MIF may regulate tumor progression on several levels. For instance, it has been shown to inhibit the activity of the p53 tumor suppressor protein (43, 46, 47). MIF is also a promoter of VEGF expression and angiogenesis, and blocking MIF activity can suppress tumor-associated vessel formation in cancer models (45, 49 - 51). In humans, MIF expression has been correlated with an increase in vessel density and enhanced risk of tumor recurrence (52). MIF may also enhance the migratory and/or invasive capabilities of tumor cells (49, 51).

The Quantikine Human MIF Immunoassay is a 3.5 hour solid-phase ELISA designed to measure human MIF in cell culture supernates, serum, and plasma. 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 the Quantikine Human MIF kit can be used to determine relative mass values for naturally occurring MIF.

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for 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 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 standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## MATERIALS PROVIDED

Description	Part #	Cat. # DMF00	Cat. # SMF00
<b>MIF Microplate</b> - 96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse monoclonal antibody against MIF.	891019	1 plate	6 plates
<b>MIF Conjugate</b> - 21 mL/vial of polyclonal antibody against MIF conjugated to horseradish peroxidase with preservatives.	891020	1 vial	6 vials
<b>MIF Standard</b> - Recombinant human MIF in a buffered protein base with preservatives; lyophilized. <b>Refer to the vial label for reconstitution volume.</b>	891021	1 vial	6 vials
<b>Assay Diluent RD1-34</b> - 11 mL/vial of a buffered protein base with preservatives.	895265	1 vial	6 vials
<b>Calibrator Diluent RD5-34</b> - 21 mL/vial of a buffered protein base with preservatives.	895828	1 vial	6 vials
<b>Wash Buffer Concentrate</b> - 21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservatives.	895003	1 vial	6 vials
<b>Color Reagent A</b> - 12.5 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	6 vials
<b>Color Reagent B</b> - 12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	6 vials
<b>Stop Solution</b> - 6 mL/vial of 2 N sulfuric acid.	895032	1 vial	6 vials
<b>Plate Covers</b> - Adhesive strips.	—	4 strips	24 strips

DMF00 contains sufficient materials to run an ELISA on one 96 well plate.

SMF00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDMF00). 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.

## STORAGE

<b>Unopened Kit</b>	Store at 2 - 8° C. Do not use past kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Diluted Wash Buffer	May be stored for up to 1 month at 2 - 8° C.*
	Stop Solution	
	Assay Diluent RD1-34	
	Calibrator Diluent RD5-34	
	Conjugate	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	
	Standard	Aliquot and store for up to 1 month at ≤ -20° C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Microplate Wells	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.*	

\*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.
- Squirrt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of  $500 \pm 50$  rpm.
- Human MIF Controls (optional; available from R&D Systems).
- **Polypropylene test tubes.**

## PRECAUTION

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

## SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20^{\circ}$  C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma, EDTA plasma, and hemolyzed samples are not suitable for use in this assay.*

## SAMPLE PREPARATION

All samples require at least a 10-fold dilution. A suggested 10-fold dilution is 30  $\mu$ L sample + 270  $\mu$ L Calibrator Diluent RD5-34.

## REAGENT PREPARATION

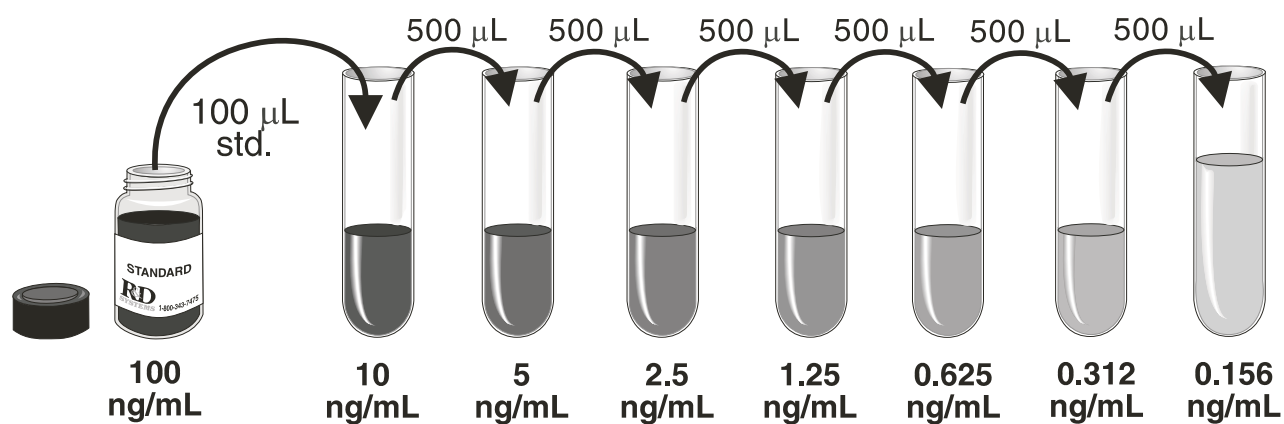
**Bring all reagents to room temperature before use.**

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of 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. Protect from light. 200  $\mu$ L of the resultant mixture is required per well.

**MIF Standard - Refer to vial label for reconstitution volume.** Reconstitute the 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, but no more than 1 hour, with gentle agitation prior to making dilutions.

**Use polypropylene tubes.** Pipette 900  $\mu$ L of Calibrator Diluent RD5-34 into the 10 ng/mL tube. Pipette 500  $\mu$ L Calibrator Diluent RD5-34 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 RD5-34 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 samples, controls, and standards be assayed in duplicate.**

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, reseal.
3. Add 100  $\mu\text{L}$  of Assay Diluent RD1-34 to each well.
4. Add 100  $\mu\text{L}$  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  $500 \pm 50$  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  $\mu\text{L}$ ) 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 200  $\mu\text{L}$  of MIF Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50  $\mu\text{L}$  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.

## ASSAY PROCEDURE SUMMARY

1. Prepare reagents, samples, and standards as instructed.



2. Add 100  $\mu\text{L}$  Assay Diluent RD1-34 to each well.



3. Add 100  $\mu\text{L}$  Standard, control, or sample\* to each well. Incubate 2 hrs. on the shaker at RT.



4. Aspirate and wash 4 times.



5. Add 200  $\mu\text{L}$  Conjugate to each well. Incubate 1 hr. on the shaker at RT.



6. Aspirate and wash 4 times.



7. Add 200  $\mu\text{L}$  Substrate Solution to each well. Incubate 30 min. **on the benchtop. Protect from light.**



8. Add 50  $\mu\text{L}$  Stop Solution to each well. Read at 450 nm within 30 min.  
 $\lambda$  correction 540 or 570 nm

\*Samples require dilution. See Sample Preparation section.

## CALCULATION OF RESULTS

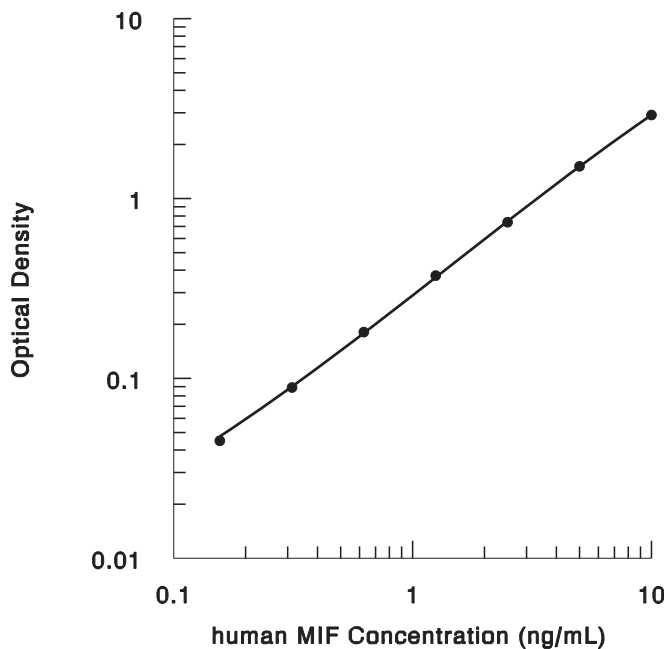
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

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 MIF 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.

Because 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	0.050 0.052 0.096	0.051	—
0.156	0.097 0.138	0.096	0.045
0.312	0.143 0.230	0.140	0.089
0.625	0.234 0.421	0.232	0.181
1.25	0.427 0.786	0.424	0.373
2.5	0.794 1.534	0.790	0.739
5	1.591 2.957	1.562	1.511
10	2.978	2.967	2.916

## 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.
- 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.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

## 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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	0.89	2.58	5.24	0.88	2.44	5.15
Standard deviation	0.04	0.17	0.20	0.08	0.16	0.26
CV (%)	4.5	6.6	3.8	9.1	6.6	5.0

## RECOVERY

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

Sample	Average % Recovery	Range
Cell culture media* (n=4)	100	91 - 108%
Serum* (n=4)	97	87 - 109%
Heparin plasma* (n=4)	102	92 - 113%

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

## LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of MIF and serially diluted with Calibrator Diluent RD5-34 to produce samples with values within the dynamic range of the assay.

		Cell culture media* (n=4)	Serum* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	98	100	102
	Range (%)	94 - 102	97 - 104	100 - 106
1:4	Average % of Expected	96	100	101
	Range (%)	94 - 97	96 - 103	97 - 106
1:8	Average % of Expected	94	101	104
	Range (%)	86 - 102	100 - 105	99 - 111
1:16	Average % of Expected	94	98	99
	Range (%)	88 - 100	90 - 101	91 - 107

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

## SENSITIVITY

Thirty-two assays were evaluated and the minimum detectable dose (MDD) of human MIF ranged from 0.007 - 0.046 ng/mL. The mean MDD was 0.017 ng/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.

## CALIBRATION

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

## SAMPLE VALUES

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

Sample Type	Mean (ng/mL)	Standard Deviation (ng/mL)	Range (ng/mL)
Serum (n=36)	11.1	2.7	5.7 - 21.7
Heparin plasma (n=36)	18.7	4.9	10.1 - 31.4

**Cell Culture Supernates** - Human peripheral blood cells ( $1 \times 10^6$  cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10  $\mu$ g/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of natural MIF.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	45.0	42.3
Stimulated	41.1	186

## SPECIFICITY

This assay recognizes recombinant and natural human MIF. The factors listed below were prepared at 100 ng/mL in Calibrator Diluent RD5-34 and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range recombinant human MIF control were assayed for interference. No significant cross-reactivity or interference was observed.

<b>Recombinant human:</b>		<b>Recombinant mouse:</b>	<b>Recombinant rat:</b>
ANG	IL-15	CT-1	CNTF
ANG-2	IL-16	CTLA-4	GDNF
AR	IL-17	Fas	IFN- $\gamma$
BDNF	Leptin	Fas Ligand	IL-1 $\alpha$
CD4	LIF	GITR Ligand	IL-1 $\beta$
CD40	NT-3	IFN- $\gamma$	IL-2
CD40 Ligand	NT-4	IL-1 $\alpha$	IL-4
CNTF	OPG	IL-1 $\beta$	IL-6
CT-1	OSM	IL-1ra	IL-10
CTLA-4	PTN	IL-2	Leptin
Epo	SCF	IL-3	MK
Fas	SLPI	IL-4	TNF- $\alpha$
GDNF	SMDF	IL-5	
GITR	TNF- $\alpha$	IL-6	
IFN- $\gamma$	TNF- $\beta$	IL-7	<b>Recombinant porcine:</b>
IL-1 $\alpha$	Tpo	IL-9	IL-1 $\alpha$
IL-1 $\beta$	TRAIL	IL-10	IL-1 $\beta$
IL-1ra	TRANCE	IL-11	IL-2
IL-2		IL-12	IL-4
IL-3		IL-12 p40	IL-6
IL-4		IL-13	IL-8
IL-5		IL-17	IL-10
IL-6		Leptin	TNF- $\alpha$
IL-7		LIF	
IL-8		OPG	
IL-9		OPN	
IL-10		OSM	
IL-11		SCF	
IL-12		TNF- $\alpha$	
IL-12 p40		Tpo	
IL-13		TRANCE	

Recombinant mouse MIF prepared at 50 ng/mL in Calibrator Diluent cross-reacts at 0.7%.

*This product and/or its use is covered by US Patent No. 6,998,238.*

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# PLATE LAYOUT

Use this plate layout as a record of standards and samples assayed.

	A	B	C	D	E	F	G	H
1								
2								
3								
4								
5								
6								
7								
8								
9								
10								
11								
12								