Quantikine® ELISA

Human CCL4/MIP-1β Immunoassay

Catalog Number DMB00
SMB00
PDMB00

For the quantitative determination of human Macrophage Inflammatory Protein 1 beta (MIP-1β) 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.

TABLE OF CONTENTS

SECTION PAGE

Manufactured and Distributed by:

USA R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

The macrophage inflammatory proteins (MIP-1α and MIP-1β) are members of the β or CC subfamily of chemokines. These two closely related proteins were initially co-purified from the conditioned media of a lipopolysaccharide-stimulated mouse macrophage cell line (1). At least seven human cDNAs that are homologous to the mouse MIP-1β, including Act-2, pAT744, H400, G-26, HIMAP, HC21, and MAD-5, have been cloned. The predicted protein products encoded by these human MIP-1β cDNAs are between 94-98% identical (2-5). There is evidence to suggest that multiple non-allelic genes for human MIP-1β exist in the human genome.

The cDNAs for human MIP-1β encode precursor proteins with a 23 amino acid (aa) residue signal peptide that is cleaved to generate the 69 aa residue, non-glycosylated mature protein. The mRNAs for MIP-1α and MIP-1β are not expressed in unstimulated cells, but the two are coordinately expressed after activation of T cells, B cells, monocytes and mast cells. In addition, both mRNAs have also been detected in macrophages associated with human atherosclerotic plaques. Mature human MIP-1α and MIP-1β are about 75% and 77% homologous, respectively, to their mouse counterparts. The mature human MIP-1α and human MIP-1β are also 70% homologous in their protein sequences. The mouse and human proteins show cross-species bioactivities (2-5).

Chemokines are small, secreted cytokines that are involved in a variety of immune and inflammatory responses, acting primarily as chemoattractants and activators of specific leukocytes. Members of each chemokine subfamily have unique as well as overlapping activities. Like other CC chemokines, both MIP-1β and MIP-1α can chemoattract monocytes (5). In addition, MIP-1α and MIP-1β can chemoattract and induce the adhesion of T lymphocytes (5). MIP-1α, but not MIP-1β, has been shown to chemoattract B cells (6), to chemoattract and degranulate eosinophils (7), to induce histamine release from basophils and mast cells, and to chemoattract basophils (8). MIP-1α has been identified as a stem cell inhibitor (SCI) that can inhibit the proliferation of hematopoietic stem cells both *in vitro* and *in vivo* (9). MIP-1β was reported to be twenty-fold less active than MIP-1α on the same cell populations (10).

Chemokine activities are mediated by G-protein-coupled seven transmembrane domain receptors (5, 11). The β chemokine receptor designated CC chemokine receptor-1 (CC CKR-1), alternately named MIP-1α/RANTES receptor, has been shown to bind MIP-1α, RANTES, MIP-1β and MCP-1 with varying affinities (12, 13). When transfected into a kidney cell line however, CC CKR-1 transduces a signal only in response to MIP-1α and RANTES. Based on functional analysis of MIP-1β, one or more additional β chemokine receptors that transduce MIP-1β signals must also exist. MIP-1α and MIP-1β have been shown to lack affinity for the erythrocyte surface chemokine receptor/Duffy antigen that binds many α as well as β chemokines (11, 14).

The Quantikine® Human CCL4/MIP-1β Immunoassay is a 3.0-4.5 hour solid phase ELISA designed to measure human MIP-1β in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human MIP-1β (Act-2 variant) and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant human MIP-1β. Results obtained using natural human MIP-1β 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 human MIP-1β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MIP-1β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MIP-1β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MIP-1β 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 MIP-1β 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.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, further dilute the samples with the appropriate 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 # DMB00	CATALOG # SMB00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human MIP-1β Microplate	890215	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MIP-1β.	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 MIP-1β Conjugate	890216	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human MIP-1β conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human MIP-1β Standard	890217	1 vial	6 vials	Recombinant human MIP-1β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1X	895121	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives. *May contain crystals. Warm to room temperature to dissolve.*	
Calibrator Diluent RD5K	895119	2 vials	12 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6O	895120	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *May contain a precipitate. Mix well before and during use. For serum/plasma samples.*	
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 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DMB00 contains sufficient materials to run an ELISA on one 96 well plate.
SMB00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDMB00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL, and not in the glass vials described in the package insert. **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Human MIP-1β Microplate	890215	50 plates
Human MIP-1β Conjugate	890216	50 vials
Human MIP-1β Standard	890217	25 vials
Calibrator Diluent RD5K	895119	100 vials
or		
Calibrator Diluent RD6O	895120	50 vials
Assay Diluent RD1X	895121	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	50 vials
Color Reagent B	895001	50 vials
Stop Solution	895032	50 vials
Plate Sealers	N/A	100 sheets
Package Insert	750126	2 booklets

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.
- Test tubes for dilution of standards and samples.
- Human MIP-1β Controls (optional; R&D Systems®, Catalog # QC20).

PRECAUTIONS

Calibrator Diluent RD6O 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. 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 ≤ -20 °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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Cell culture supernate samples may require up to a 20-fold dilution into Calibrator Diluent RD5K. A suggested 20-fold dilution is 25 µL of sample + 475 µL of Calibrator Diluent RD5K.

REAGENT PREPARATION

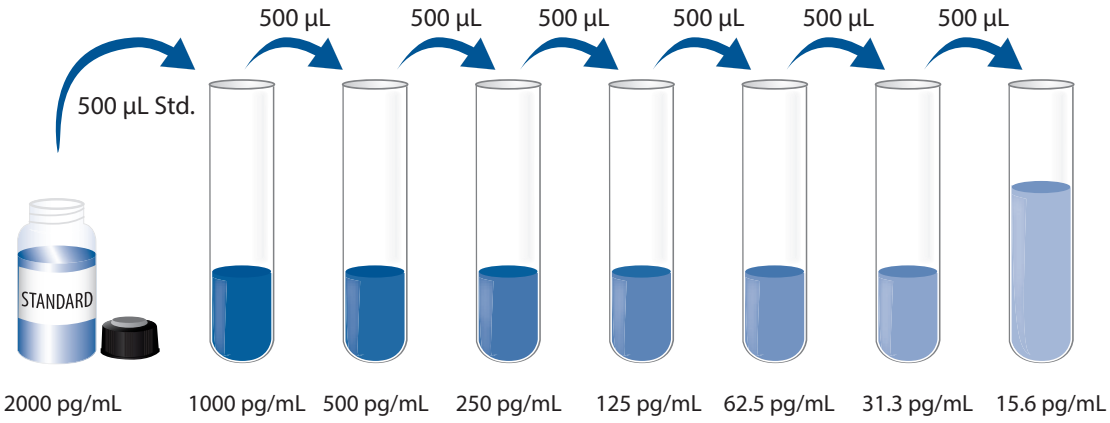
Bring all reagents to room temperature before use.

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

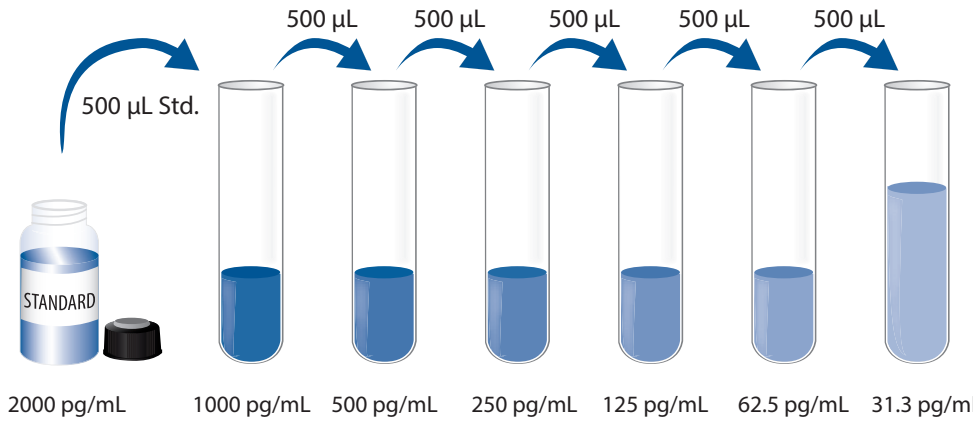
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human MIP-1β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MIP-1β Standard with Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6O (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

For Cell Culture Supernate Samples - Pipette 500 µL of Calibrator Diluent RD5K into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL dilution serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 pg/mL).



For Serum/Plasma Samples - Pipette 500 µL of Calibrator Diluent RD6O into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human MIP-1β Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD6O 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, controls, and samples 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, and reseal.
3. RD1X may contain crystals. Warm to room temperature and mix well before use.
   For Cell Culture Supernate Samples: Add 50 µL of Assay Diluent RD1X to each well.
   For Serum/Plasma Samples: Add 100 µL of Assay Diluent RD1X to each well.
4. **For Cell Culture Supernate Samples:** Add 200 µL of standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 1.25 hours at room temperature.
   For Serum/Plasma Samples: Add 150 µL of standard, control, or sample per well. 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 twice for a total of three washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. **After 30 seconds, empty wells by aspiration or decanting.** 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 MIP-1β Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1.25 hours at room temperature.
   For Serum/Plasma Samples: Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **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 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.).

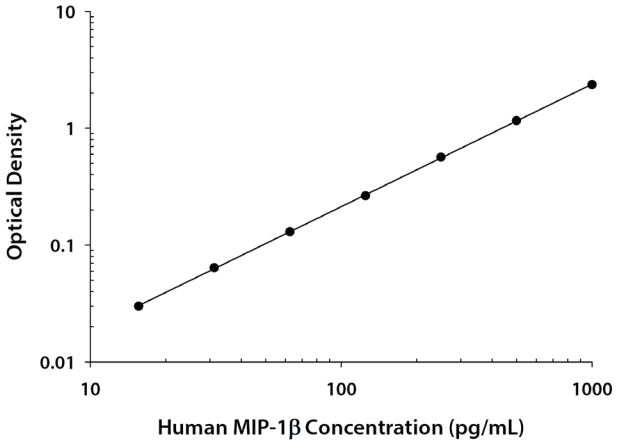
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human MIP-1β 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

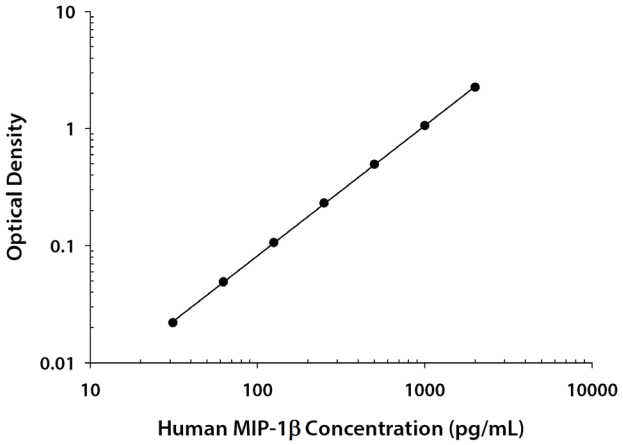
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.031 0.032	0.032	—
15.6	0.062 0.061	0.062	0.030
31.3	0.094 0.097	0.096	0.064
62.5	0.161 0.164	0.162	0.130
125	0.297 0.294	0.296	0.264
250	0.591 0.601	0.596	0.564
500	1.177 1.206	1.192	1.160
1000	2.408 2.381	2.394	2.362

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.040 0.035	0.038	—
31.3	0.061 0.060	0.060	0.022
62.5	0.089 0.085	0.087	0.049
125	0.145 0.144	0.144	0.106
250	0.267 0.271	0.269	0.231
500	0.537 0.527	0.532	0.494
1000	1.103 1.091	1.097	1.059
2000	2.354 2.223	2.288	2.250

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.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	23.3	98.8	428	31.4	118	476
Standard deviation	1.10	3.80	17.3	3.70	9.90	28.0
CV (%)	4.7	3.8	4.0	11.8	8.4	5.9

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	51.3	208	932	58.8	220	949
Standard deviation	4.60	7.50	30.2	5.70	15.6	70.5
CV (%)	9.0	3.6	3.2	9.7	7.1	7.4

RECOVERY

The recovery of human MIP-1β spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	107	81-117%
Serum (n=5)	99	88-113%
EDTA plasma (n=5)	100	84-112%
Heparin plasma (n=5)	99	90-112%
Citrate plasma (n=5)	99	87-116%

SENSITIVITY

Using Calibrator Diluent RD5K the minimum detectable dose (MDD) of human MIP-1β is typically less than 4.0 pg/mL. Using Calibrator Diluent RD6O the MDD is typically less than 11.0 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.

LINEARITY

To assess linearity of the assay, samples were spiked with high concentrations of human MIP-1β and diluted with the calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)
1:2	Average % of Expected	97	95	94	93	95
	Range (%)	92-100	90-100	86-99	84-103	89-98
1:4	Average % of Expected	96	93	91	91	93
	Range (%)	91-100	87-100	85-96	83-99	89-101
1:8	Average % of Expected	94	94	93	94	96
	Range (%)	85-103	87-102	84-111	83-105	88-108
1:16	Average % of Expected	91	97	95	99	98
	Range (%)	81-99	90-110	88-107	86-106	86-115

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human MIP-1β (Act-2 variant) produced at R&D Systems®.

SAMPLE VALUES

Serum - Samples from apparently healthy volunteers were evaluated for the presence of human MIP-1β in this assay. No medical histories were available for the donors used in this study.

Condition	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=37)	80	94	ND-212
EDTA plasma (n=37)	30	40	ND-86
Heparin plasma (n=37)	40	65	ND-94
Citrate plasma (n=37)	33	35	ND-119

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate and stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed on day 1 and assayed for levels of human MIP-1β.

Condition	Day 1 (pg/mL)
Unstimulated	231
Stimulated	19,058

SPECIFICITY

This assay recognizes natural and recombinant human MIP-1β.

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 MIP-1β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
AR
CNTF
β-ECGF
EGF
Epo
FGF acidic
FGF basic
FGF-4
FGF-5
FGF-6
G-CSF
GM-CSF
gp130
GROα
GROβ
GROγ
HB-EGF
HGF
IFN-γ
IGF-I
IGF-II
IL-1α
IL-1β
IL-1ra
IL-1 RI
IL-1 RII
IL-2
IL-2 Rα
IL-3
IL-3 Rα
IL-3 Rβ
IL-4
IL-5
IL-5 Rα
IL-6
IL-6 R
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-13
KGF
LAP (TGF-β1)
LIF
MCP-1
M-CSF
MIP-1α
β-NGF
OSM
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
PTN
RANTES
SCF
SLPI
TGF-α
TGF-β1
TGF-β3
TGF-β RII
TNF-α
TNF-β
TNF RI
TNF RII
VEGF

Recombinant mouse:

GM-CSF
IL-1α
IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
LIF
MIP-1α
MIP-1β
SCF
TNF-α

Other recombinants:

amphibian TGF-β5
chicken TGF-β3

Natural proteins:

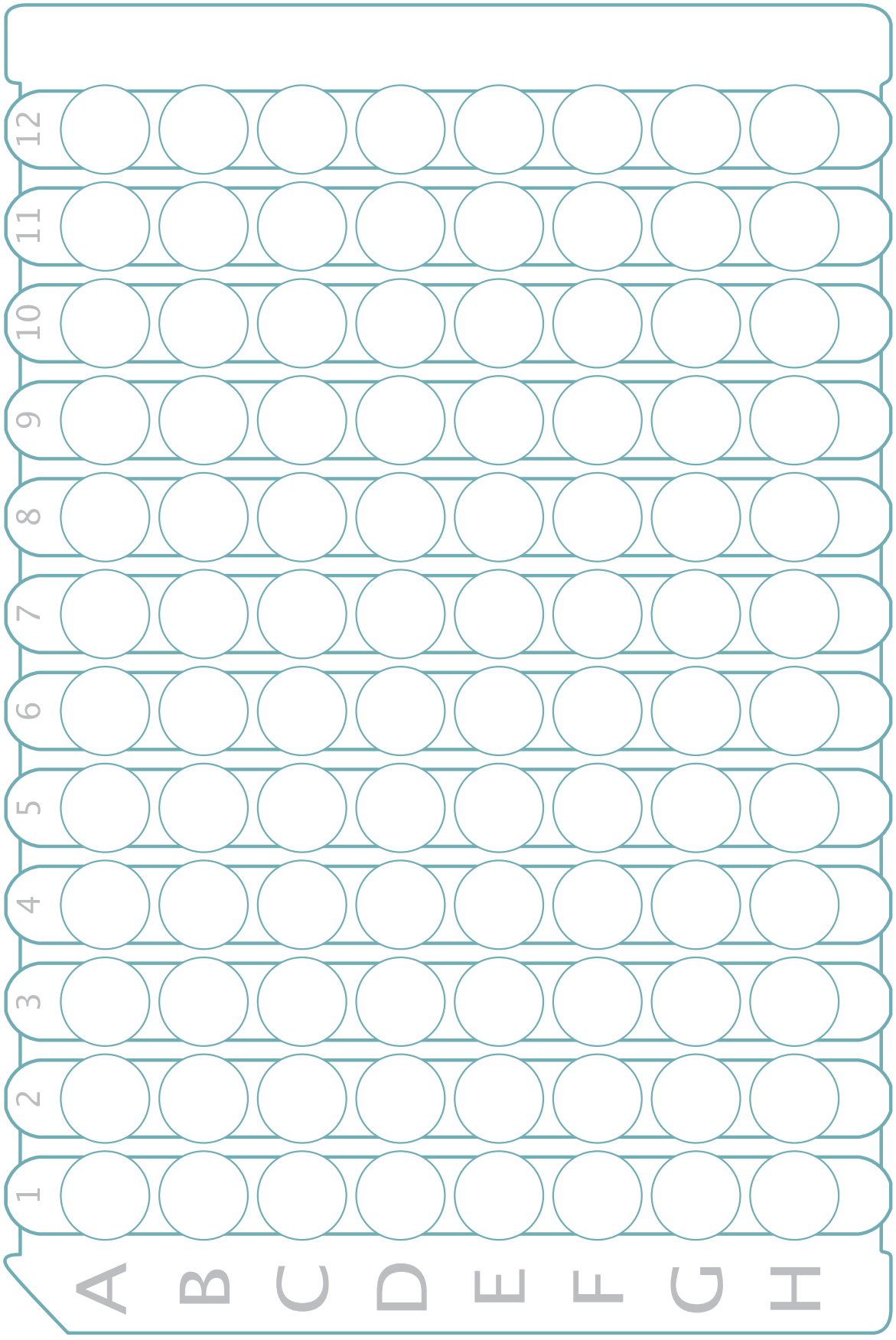
mouse EGF
bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1

REFERENCES

1. Wolpe, S.D. and A. Cerami (1989) FASEB J. **3**:2565.
2. Oppenheim, J.J. *et al.* (1991) Annu. Rev. Immunol. **9**:617.
3. Schall, T.J. (1991) Cytokine **3**:165.
4. Miller, M.D. and M.S. Krangel (1992) Critical Rev. Immunol. **12**:17.
5. Schall, T.J. (1994) in *The Cytokine Handbook*, 2nd edition, A. Thomson ed., Academic Press, New York, p. 419.
6. Schall, T.J. *et al.* (1993) J. Exp. Med. **177**:1821.
7. Rot, A. *et al.* (1992) J. Exp. Med. **176**:1489.
8. Alam, R. *et al.* (1992) J. Exp. Med. **176**:781.
9. Graham, G.J. *et al.* (1990) Nature **344**:442.
10. Graham, G.J. *et al.* (1993) Cell Growth Diff. **4**:137.
11. Murphy, P.M. (1994) Annu. Rev. Immunol. **12**:593.
12. Neote, K. *et al.* (1993) Cell **72**:415.
13. Gao, J.L. *et al.* (1993) J. Exp. Med. **177**:1421.
14. Neote, K. *et al.* (1993) J. Biol. Chem. **268**:12247.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES