Quantikine® ELISA

Human CCL7/MCP-3 Immunoassay

Catalog Number DCC700

For the quantitative determination of human Monocyte Chemotactic Protein 3 (MCP-3) 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

Monocyte Chemotactic Protein-3 (MCP-3), also known as CCL7, was originally purified from stimulated MG-63 human osteosarcoma cells as a protein chemotactic for monocytes (1). MCP-3 is capable of recruiting and activating most types of leukocytes, however, making it one of the most pluripotent known chemokines (2-11). MCP-3 has been implicated in immunity-associated tumor rejection, and in a number of pathologies involving immune response (12-19). For a review of the MCP family of chemokines, see reference 20.

The MCP-3 gene is located within the CC chemokine cluster on chromosome 17 (21). The corresponding cDNA encodes a 99 amino acid (aa) precursor protein with a 23 aa signal sequence that is cleaved to generate the 76 aa mature chemokine (2). The predicted molecular mass of non-glycosylated MCP-3 is approximately 9 kDa, but differential *N*- and *O*-glycosylation results in variable molecular weights greater than 11 kDa (1, 22, 23). Unlike many chemokines, MCP-3 is impervious to cleavage and inactivation by dipeptidyl peptidase IV, but it can be processed by MMP-2 (gelatinase A), resulting in a 71 aa protein that binds its receptors without activity (24, 25).

mRNA expression of MCP-3 is constitutive in platelets and has been induced by various inflammatory cytokines in monocytes, mononuclear cells, fibroblasts, endothelial cells, airway smooth muscle cells (ASMCs), THP-1 myelomonocytic leukemia cells, MG-63 osteosarcoma cells, and U937 promonocytic leukemia cells (1, 23, 26-29). Inflammatory cytokine-dependent expression is inhibited by IL-4, IL-10, and IL-13 in monocytes and by dexamethasone in ASMCs (26, 28).

MCP-3 binds and signals via the CC chemokine receptors CCR1, CCR2A, CCR2B, CCR3, and appears to be an antagonist for CCR5 (30). This wide range of receptor recognition accounts for the extensive number of cell types this chemokine can influence. *In vitro* chemotaxis in response to MCP-3 has been exhibited by freshly isolated $CD4^+$ and $CD8^+$ lymphocytes, T cell clones, monocytes, and dendritic cells at minimal effective concentrations (MECs) of less than 10 ng/mL (5). MECs of MCP-3 between 10 and 30 ng/mL induce chemotaxis of IL-2 stimulated NK cells, NK cell clones, and neutrophils (5, 6, 11). MCP-3 at concentrations greater than 30 ng/mL stimulates migration of eosinophils and basophils (7, 8). In addition, MCP-3 stimulates the release of MMP-9 (gelatinase B) and granzyme A in monocytes, T-lymphocytes, and NK cells, which presumably enables degradation of the extracellular matrix and subsequent tissue infiltration (3, 4, 6).

Low concentrations of MCP-3 inhibit, or partially inhibit, infection by some HIV-1 strains, while higher concentrations enhance viral replication (30-32). The mechanisms for these effects are unclear, but undoubtedly there is complex interplay involving MCP-3, CCR2A, CCR3, and CCR5. In addition to binding MCP-3, CCR2A, CCR3, and CCR5 are co-receptors for HIV-1 entry (30-32). MCP-3 also appears to induce immunity-associated tumor regression. Mastocytoma cells capable of growing in syngenic hosts are rejected when transfected with MCP-3, showing elevated accumulation of MCP-3 target cells in tumor associated tissue (12, 13). In addition, MCP-3 may play a role in autoimmunity and other inflammatory conditions. Levels are elevated in acute and chronic multiple sclerosis (MS) lesions, and MCP-3 receptor expression is up-regulated in the central nervous system of MS patients (14). Asthma, chronic sinusitis, ulcerative colitis, bilial cirrhosis, and vernal keratoconjunctivitis also demonstrate elevated levels of MCP-3 (15-19).

The Quantikine Human CCL7/MCP-3 Immunoassay is a 4.5 hour solid phase ELISA designed to measure MCP-3 levels in cell culture supernates, serum, and plasma. It contains *E. coli*- expressed recombinant human MCP-3 and antibodies raised against the recombinant factor. Results obtained for naturally occurring human MCP-3 showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that this kit can be used to determine relative mass values for natural human MCP-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MCP-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MCP-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human MCP-3 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 MCP-3 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, dilute the samples with the appropriate 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.
- 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.

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. Please refer to the MSDS on our website prior to use.

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
Human MCP-3 Microplate	892285	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MCP-3.	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 MCP-3 Conjugate	892286	21 mL of monoclonal antibody specific for human MCP-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human MCP-3 Standard	892287	Recombinant human MCP-3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-24	895325	21 mL of a buffered protein base with preservatives. *Use undiluted for serum/plasma samples. Use diluted 1:2 for cell culture supernate samples.*	
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	895032	6 mL of 2 N sulfuric 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.
- **Polypropylene** test tubes for dilution of standards.
- Human MCP-3 Controls (optional; R&D Systems, Catalog # QC119).

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 or 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 ≤ -20 °C. Avoid repeated freeze-thaw cycles.

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

REAGENT PREPARATION

Bring all reagents to room temperature before use.

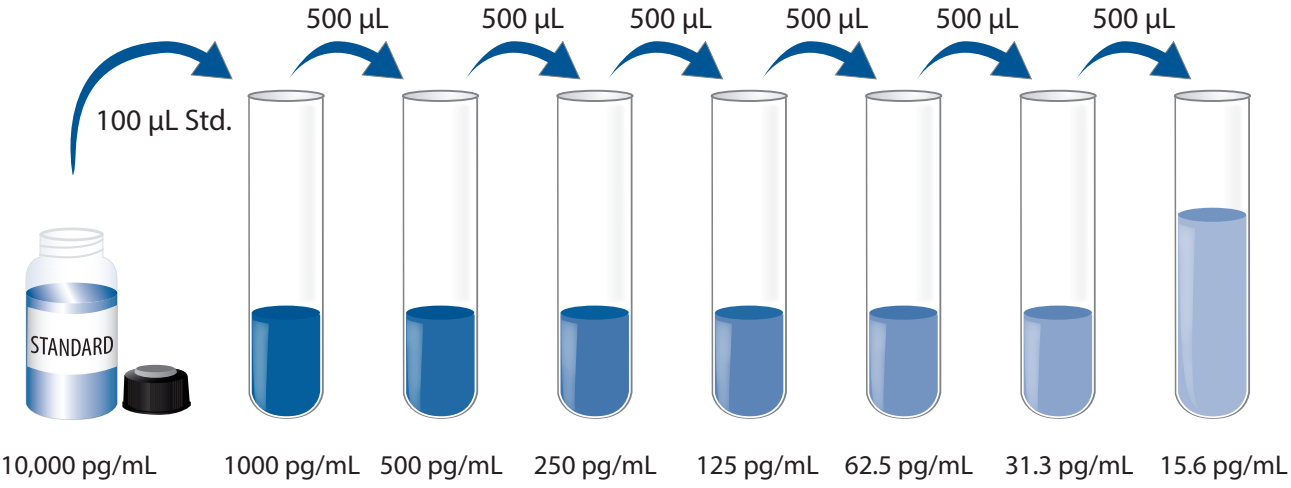
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD5-24 (diluted 1:2) - **For cell culture supernate samples only.** Add 10 mL of Calibrator Diluent RD5-24 to 10 mL deionized or distilled water to prepare 20 mL of Calibrator Diluent RD5-24 (diluted 1:2).

Human MCP-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MCP-3 Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 pg/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.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-24 (diluted 1:2) (*for cell culture supernate samples*) or Calibrator Diluent RD5-24 (*for serum/plasma samples*) into the 1000 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1000 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent 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, 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, and reseal.
3. Add 100 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 3 hours at room temperature. 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 MCP-3 Conjugate to each well. Cover with a new adhesive strip. Incubate for 1 hour at room temperature.
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. **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.

CALCULATION OF RESULTS

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

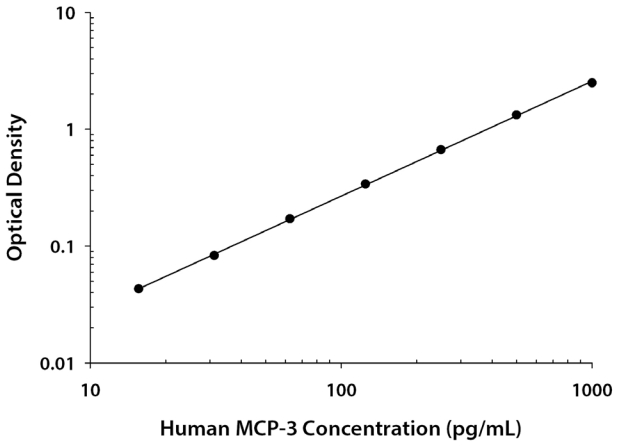
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human MCP-3 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

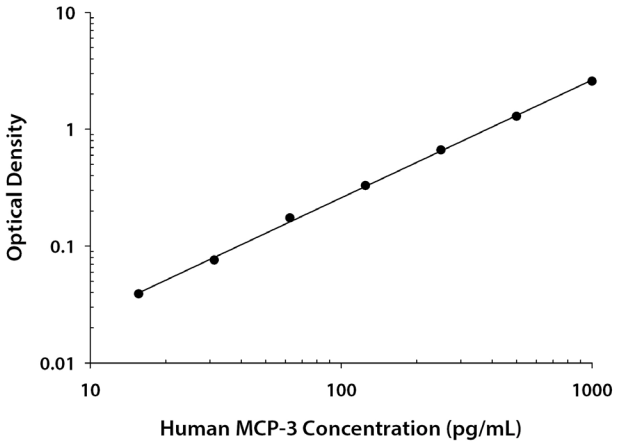
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.006 0.006	0.006	—
15.6	0.046 0.051	0.049	0.043
31.3	0.085 0.092	0.089	0.083
62.5	0.173 0.180	0.177	0.171
125	0.337 0.354	0.346	0.340
250	0.658 0.688	0.673	0.667
500	1.331 1.335	1.333	1.327
1000	2.435 2.554	2.495	2.489

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.006 0.006	0.006	—
15.6	0.044 0.046	0.045	0.039
31.3	0.081 0.083	0.082	0.076
62.5	0.176 0.183	0.180	0.174
125	0.328 0.343	0.336	0.330
250	0.668 0.669	0.669	0.663
500	1.297 1.354	1.326	1.320
1000	2.569 2.579	2.574	2.568

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)	136	274	506	138	277	524
Standard deviation	6.24	8.35	18.1	6.87	14.2	24.3
CV (%)	4.6	3.0	3.6	5.0	5.1	4.6

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)	140	278	560	152	297	575
Standard deviation	5.94	10.7	20.2	10.2	19.9	37.7
CV (%)	4.2	3.8	3.6	6.7	6.7	6.6

RECOVERY

The recovery of human MCP-3 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	106	92-115%
Serum (n=4)	94	86-110%
EDTA plasma (n=4)	101	88-111%
Heparin plasma (n=4)	98	85-114%

SENSITIVITY

Eighty-four assays were evaluated and the minimum detectable dose (MDD) of human MCP-3 ranged from 0.29-8.52 pg/mL. The mean MDD was 1.01 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human MCP-3 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=3)	Cell culture media (n=4)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	102	104	105	105	104
	Range (%)	100-104	103-106	100-112	101-109	102-107
1:4	Average % of Expected	98	103	107	105	108
	Range (%)	98-99	99-109	97-115	102-108	105-113
1:8	Average % of Expected	98	100	104	103	104
	Range (%)	97-99	97-107	99-109	99-108	100-113
1:16	Average % of Expected	99	99	108	105	103
	Range (%)	98-100	95-103	97-117	100-113	100-109

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human MCP-3 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Fifty serum samples and thirty-five EDTA plasma samples from apparently healthy volunteers were evaluated for the presence of human MCP-3 in this assay. All samples read less than the low standard, 15.6 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates:

Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the culture supernate were removed on days 1 and 5 and assayed for levels of human MCP-3.

Condition	Day 1 (pg/mL)	Day 2 (pg/mL)
Unstimulated	ND	ND
Stimulated	2051	3099

ND=Non-detectable

MG-63 human osteosarcoma cells were cultured in DMEM supplemented with 10% fetal bovine serum and grown to confluence. An aliquot of the cell culture supernate was removed, assayed for human MCP-3, and measured 90 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human MCP-3.

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 MCP-3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

6Ckine
CTACK
Eotaxin
Eotaxin-3
HCC-1
HCC-4
I-309
MCP-1
MCP-2
MCP-4
MDC
MIP-1α
MIP-1β
MIP-1δ
MIP-3α
MIP-3β
MPIF-1
PARC
RANTES
TARC
TECK

Recombinant mouse:

6Ckine
CTACK
Eotaxin
JE/MCP-1
MARC
MCP-5
MDC
MIP-1α
MIP-1β
MIP-1γ
MIP-3α
MIP-3β
RANTES
TARC
TECK
TCA-3

Recombinant rat:

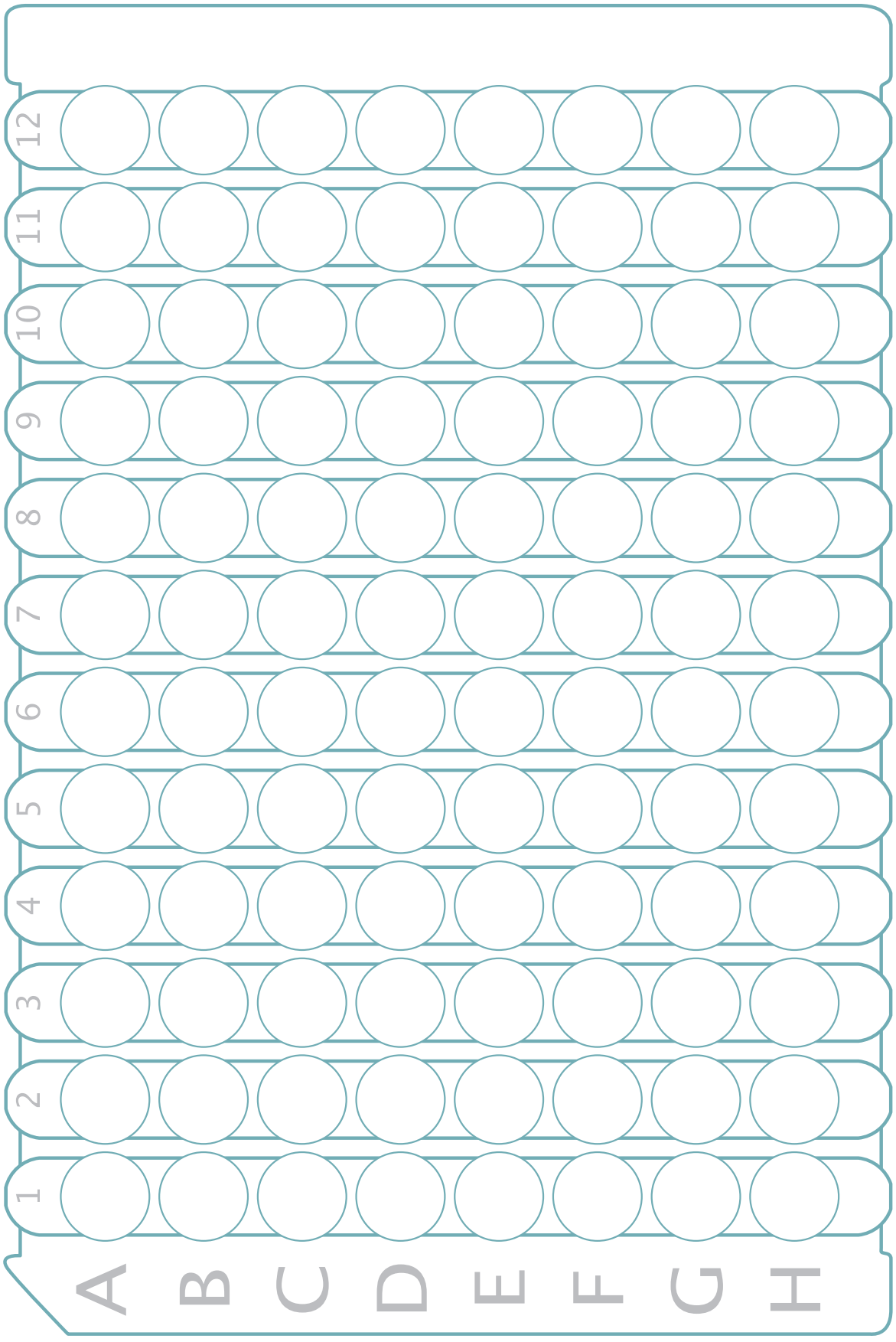
MIP-3α

REFERENCES

1. Van Damme, J. *et al.* (1992) J. Exp. Med. **176**:59.
2. Uguccioni, M. *et al.* (1995) Eur. J. Immunol. **25**:64.
3. Sozzani, S. *et al.* (1994) Biochem. Biophys. Res. Commun. **199**:761.
4. Taub, D.D. *et al.* (1995) J. Clin. Invest. **95**:1370.
5. Allavena, P. *et al.* (1994) Eur. J. Immunol. **24**:3233.
6. Loetscher, P. *et al.* (1996) J. Immunol. **156**:322.
7. Noso, N. *et al.* (1994) Biochem. Biophys. Res. Commun. **200**:1470.
8. Elsner, J. *et al.* (1996) Eur. J. Immunol. **26**:1919.
9. Weber, M. *et al.* (1995) J. Immunol. **154**:4166.
10. Alam, R. *et al.* (1994) J. Immunol. **153**:3155.
11. Xu, L.L. *et al.* (1995) Eur. J. Immunol. **25**:2612.
12. Fioretti, F. *et al.* (1998) J. Immunol. **161**:342.
13. Wetzel, K. *et al.* (2001) J. Gene Med. **3**:326.
14. McManus, C. *et al.* (1998) J. Neuroimmunol. **86**:20.
15. Miotto, D. *et al.* (2001) J. Allergy Clin. Immunol. **107**:664.
16. Uguccioni, M. *et al.* (1999) Am. J. Pathol. **155**:331.
17. Tsuneyama, K. *et al.* (2001) J. Pathol. **193**:102.
18. Wright, E.D. *et al.* (1998) J. Otolaryngol. **27**:281.
19. Abu El-Asrar, A.M. *et al.* (2000) Br. J. Ophthalmol. **84**:1360.
20. Van Collie, E. *et al.* (1999) Cytokine Growth Factor Rev. **10**:61.
21. Opdenakker, G. *et al.* (1994) Genomics **21**:403.
22. Opdenakker, G. *et al.* (1993) Biochem. Biophys. Res. Commun. **191**:535.
23. Minty, A. *et al.* (1993) Eur. Cytokine Netw. **4**:99.
24. Van Damme, J. *et al.* (1999) Chem. Immunol. **72**:42.
25. McQuibban, G.A. *et al.* (2000) Science **289**:1202.
26. Polentarutti, N. *et al.* (1997) Eur. Cytokine Netw. **8**:271.
27. Menten, P. *et al.* (1999) Eur. J. Immunol. **29**:678.
28. Pype, J.L. *et al.* (1999) Am. J. Respir. Cell Mol. Biol. **21**:528.
29. Power, C.A. *et al.* (1995) Cytokine **7**:479.
30. Blanpain, C. *et al.* (1999) Blood **94**:1899.
31. Greco, G. *et al.* (1999) J. Gen. Virol. **80**:2369.
32. Schols, D. *et al.* (1997) J. Virol. **71**:7300.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES