Quantikine® ELISA

Human CXCL9/MIG Immunoassay

Catalog Number DCX900

For the quantitative determination of human Monokine Induced by Gamma Interferon (MIG) 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

Monokine Induced by Gamma Interferon (MIG), also known as CXCL9, was originally identified through the screening of a cDNA library prepared from lymphokine-activated RAW 264.7 mouse macrophages (1). Human MIG was subsequently cloned using mouse MIG cDNA as a probe (2). The human MIG gene is located on chromosome four (3), and the corresponding cDNA encodes a 125 amino acid (aa) precursor protein with a 22 aa signal sequence that is cleaved to yield the 103 aa mature protein (2). Both the carboxy- and amino-terminal residues are prone to proteolytic cleavage, which results in size heterogeneity of both natural and recombinant preparations (4, 5). MIG, along with IP-10/CXCL10 and I-TAC/CXCL11, is a member of a CXC chemokine subfamily that lacks an ELR (Glu-Leu-Arg) motif in front of the first cysteine. Whereas ELR^+ CXC chemokines attract neutrophils and promote angiogenesis via CXCR1 and CXCR2, ELR^- CXC chemokines recruit activated lymphocytes and retard angiogenesis via CXCR3 (4, 6, 7).

CXCR3 is a seven transmembrane G-protein coupled receptor expressed on activated Th1, but not Th2 lymphocytes (7-10). CXCR3 expression has also been observed in monocytes, endothelial cells, eosinophils, malignant B cells, melanoma cells, $CD34^+$ hematopoeitic progenitors, and neurons (7, 10-16). MIG is a natural antagonist for CCR3, thus contributing further to a Th1-dominated immune response by inhibiting Th2 lymphocyte migration and calcium flux in response to the CCR3 ligands eotaxin, eotaxin-2, MCP-2, MCP-3, MCP-4, and RANTES (17).

Expression of MIG is induced by the prototypical Th1 cytokine, IFN-γ, and is dramatically enhanced by addition of TNF-α (1, 18, 19). A wide range of cells and tissues exhibit IFN-γ-dependent MIG production including monocytes/macrophages, neutrophils, keratinocytes, endothelial cells, epithelial cells, astrocytes, thyrocytes, liver, and kidney (1, 4, 20-29). As a practical test for judging vaccine efficacy, MIG production has proven to be a sensitive measure of antigen-specific IFN-γ production (30).

IFN-γ-dependent production of MIG by endothelial cells is inhibited by nitric oxide (NO) donors such as sodium nitroprusside and peroxisome proliferator-activated receptor-γ activators such as 15-deoxy-PGJ_2, and lysophosphatidylcholine (a major component of low-density lipoprotein) (23, 31, 32). These observations help explain the partial efficacy of NO donors in the treatment of glomerulonephritis, the anti-inflammatory properties of 15-deoxy-PGJ_2, and the appearance of a Th1-Th2 switch observed in hypercholesterolemia (23, 31, 32). MIG activity may be down-regulated post-translationally by dipeptidyl peptidase IV cleavage, thus producing a chemokine with no chemotactic activity yet leaving the angiostatic activity intact (5).

In mouse models, MIG is important in anti-viral host defense and can induce T cell-dependent tumor regression (33-41). Not surprisingly, MIG has been implicated in pathologies characterized by the accumulation of activated Th1 lymphocytes. These include acute allograft rejection, glomerulonephritis, autoimmunity, rheumatoid arthritis, atherosclerosis, psoriasis, and allergic contact dermatitis (20, 22-24, 42-56).

The Quantikine Human CXCL9/MIG Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human MIG in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human MIG and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human MIG showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human MIG.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MIG has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MIG present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MIG 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 MIG 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.
- Sample values increase over time when kept at 2-8 °C or room temperature. Separate serum or plasma from samples as quickly as possible and assay immediately or aliquot and freeze at ≤ -20 °C. Variations in sample collection, processing, and storage may cause sample value differences.
- 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.
- 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.

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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human MIG Microplate	892234	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MIG.	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.*
Human MIG Standard	892236	Recombinant human MIG in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C in a manual defrost freezer for up to 1 month.* Avoid repeated freeze-thaw cycles.
Human MIG Conjugate	892235	21 mL of polyclonal antibody specific for human MIG conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 for cell culture supernate samples. Use diluted 1:2 for serum/plasma 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 and samples.
- Human MIG Controls (optional; R&D Systems, Catalog # QC123).

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.

SAMPLE COLLECTION & STORAGE

Sample values increase with extended storage (> 8 hours) at temperatures ≥ 2-8 °C.

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.

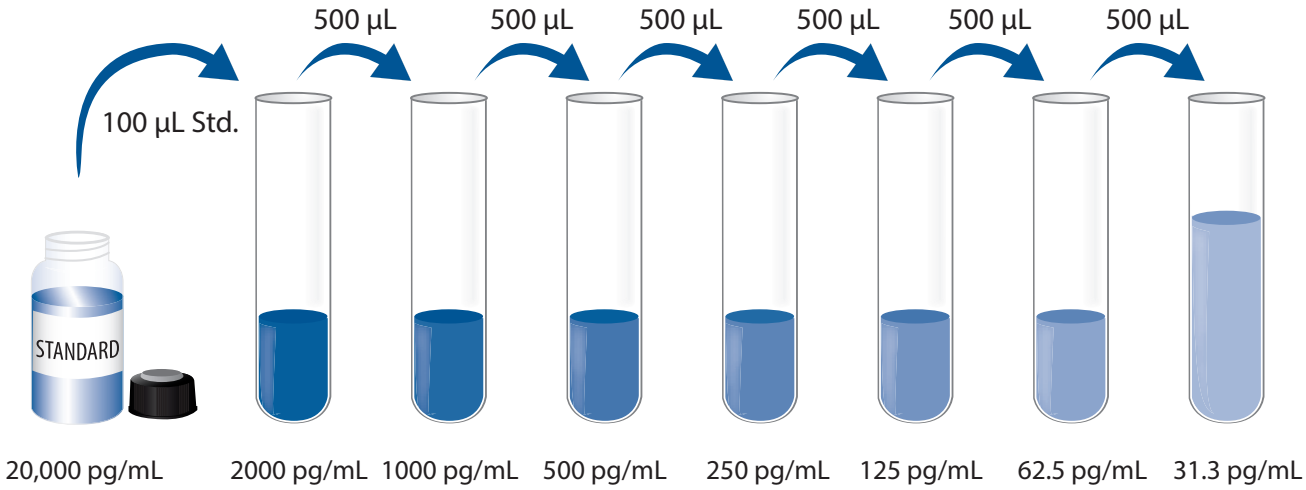
Calibrator Diluent RD5P (diluted 1:5) - **For cell culture supernate samples.** Add 10 mL of RD5P Concentrate to 40 mL of deionized or distilled water to prepare 50 mL of RD5P (diluted 1:5).

Calibrator Diluent RD5P (diluted 1:2) - **For serum/plasma samples.** Add 10 mL of RD5P Concentrate to 10 mL of deionized or distilled water to prepare 20 mL of RD5P (diluted 1:2).

Human MIG Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MIG Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard by inversion or brief vortex for 5-10 seconds to ensure complete reconstitution and then allow the standard to sit for a minimum of 15 minutes prior to making dilutions.

Note: *Do not use rocker or extended vortexing.*

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD5P (diluted 1:2) (*for serum/plasma samples*) into the 2000 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 2000 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 standards, samples, and controls 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 100 µL of Standard, sample* or control 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 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 MIG Conjugate to each well. Cover with a new adhesive strip. 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 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 if 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.

*Cell culture supernate samples may require dilution in Calibrator Diluent RD5P (1X).

CALCULATION OF RESULTS

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

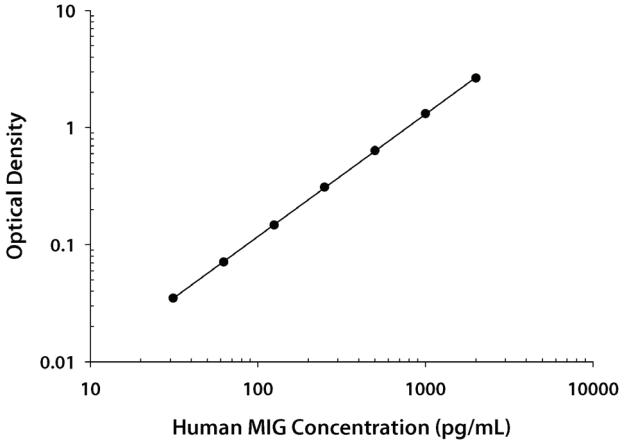
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human MIG concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

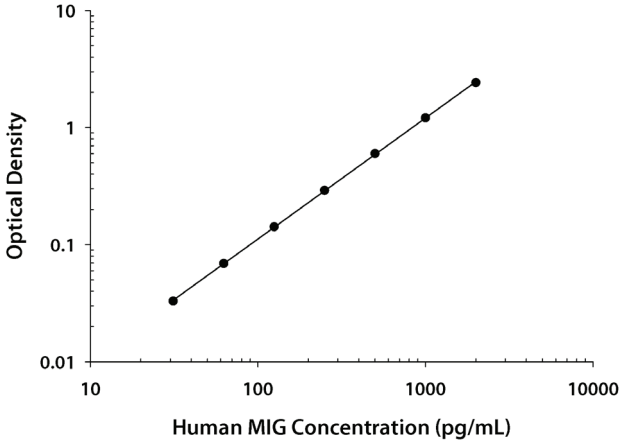
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.021 0.021	0.021	—
31.3	0.055 0.057	0.056	0.035
62.5	0.091 0.093	0.092	0.071
125	0.169 0.167	0.168	0.147
250	0.330 0.330	0.330	0.309
500	0.663 0.651	0.657	0.636
1000	1.332 1.340	1.336	1.315
2000	2.658 2.672	2.665	2.644

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.023	0.023	—
31.3	0.056 0.055	0.056	0.033
62.5	0.094 0.089	0.092	0.069
125	0.157 0.173	0.165	0.142
250	0.302 0.326	0.314	0.291
500	0.616 0.630	0.623	0.600
1000	1.217 1.245	1.231	1.208
2000	2.444 2.448	2.446	2.423

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)	235	700	1358	242	707	1391
Standard deviation	9.5	25.1	24.3	20.8	50.9	83.6
CV (%)	4.0	3.6	1.8	8.6	7.2	6.0

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)	253	817	1603	245	723	1427
Standard deviation	9.9	26.6	49.6	12.8	59.4	88.1
CV (%)	3.9	3.3	3.1	5.2	8.2	6.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	92	86-98%
Serum (n=5)	108	101-113%
EDTA plasma (n=5)	100	95-110%
Heparin plasma (n=5)	109	101-114%

SENSITIVITY

Thirty-seven assays were evaluated and the minimum detectable dose (MDD) of human MIG ranged from 1.37-11.31 pg/mL. The mean MDD was 3.84 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 spiked with high concentrations of human MIG were serially diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=5)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	105	104	102	99
	Range (%)	103-108	102-108	99-106	97-105
1:4	Average % of Expected	106	105	101	103
	Range (%)	103-110	103-107	97-103	100-106
1:8	Average % of Expected	104	105	100	104
	Range (%)	100-111	102-110	94-107	101-109
1:16	Average % of Expected	106	110	103	103
	Range (%)	98-113	100-124	95-110	96-108

CALIBRATION

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

SAMPLE VALUES

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

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=40)	64.4	65	ND-199
EDTA plasma (n=23)	56.7	62	ND-179
Heparin plasma (n=25)	62.0	68	ND-209

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 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. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural human MIG.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	445
Stimulated	77.5	12,290

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human MIG.

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

Recombinant human:

ENA-78
BLC/BCA-1
BRAK
GCP-2
GROα
GROβ
GROγ
IL-8
IL-8, endothelial cell-derived
I-TAC
IP-10
NAP-2
SDF-1α
SDF-1β

Recombinant mouse:

BLC/BCA-1
CRG-2 (IP-10)
GCP-2
KC
MIG

REFERENCES

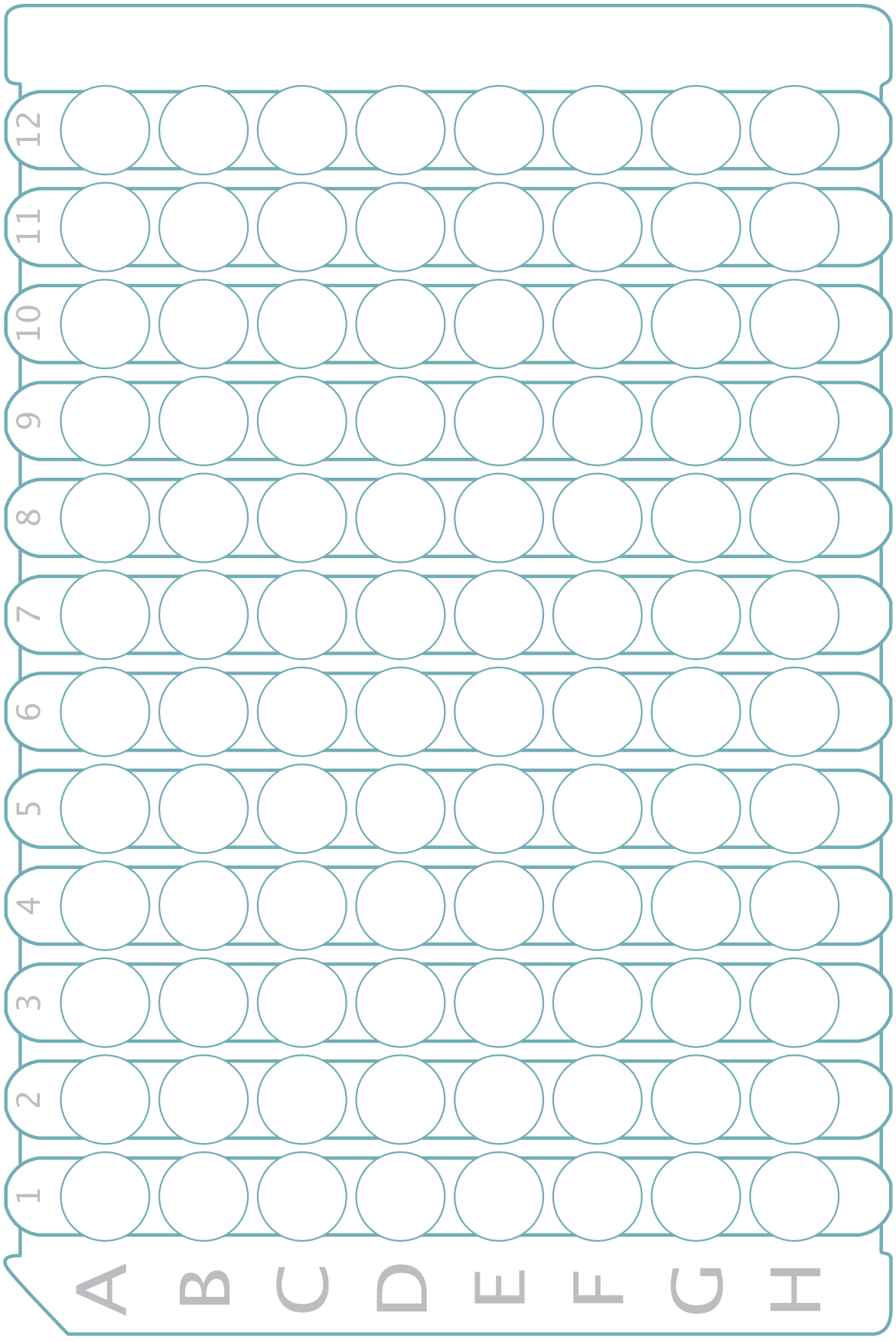
1. Farber, J.M. (1990) Proc. Natl. Acad. Sci. USA **87**:5238.
2. Farber, J.M. (1993) Biochem. Biophys. Res. Commun. **192**:223.
3. Lee H.H. and J.M. Farber (1996) Cytogenet Cell Genet. **74**:255.
4. Liao, F. *et al.* (1995) J. Exp. Med. **182**:1301.
5. Proost, P. *et al.* (2001) Blood **98**:3554.
6. Loetscher, M. *et al.* (1996) J. Exp. Med. **184**:963.
7. Romagnani, P. *et al.* (2001) J. Clin. Invest. **107**:53.
8. Bonecchi, R. *et al.* (1998) J. Exp. Med. **187**:129.
9. Sallusto, F. *et al.* (1998) J. Exp. Med. **187**:875.
10. Qin, S. *et al.* (1998) J. Clin. Invest. **101**:746.
11. Jinquan, T. *et al.* (2000) J. Immunol. **165**:1548.
12. Salcedo, R. *et al.* (2000) FASEB J. **14**:2055.
13. Trentin, L. *et al.* (1999) J. Clin. Invest. **104**:115.
14. Robledo, M.M. *et al.* (2001) J. Biol. Chem. **276**:45098.
15. Jinquan, T. *et al.* (2000) Blood **96**:1230.
16. Xia, M.Q. *et al.* (2000) J. Neuroimmunol. **108**:227.
17. Loetscher, M. *et al.* (1996) J. Biol. Chem. **276**:2986.
18. Wright, T.M. and J.M. Farber (1991) J. Exp. Med. **173**:417.
19. Wong, P. *et al.* (1994) Mol. Cell Biol. **14**:914.
20. Garcia-Lopez, M.A. *et al.* (2001) J. Clin. Endocrinol. Metab. **86**:5008.
21. Itoh, Y. *et al.* (2001) Scand. J. Gastroenterol. **36**:1344.
22. Park, J.W. *et al.* (2001) J. Immunol. **166**:3763.
23. Romagnani, P. *et al.* (2002) J. Am. Soc. Nephrol. **13**:53.
24. Romagnani, P. *et al.* (1999) J. Am. Soc. Nephrol. **10**:2518.
25. Gasperini, S. *et al.* (1999) J. Immunol. **162**:4928.
26. Goebeler, M. *et al.* (2001) Am. J. Pathol. **158**:431.
27. Albanesi, C. *et al.* (2000) J. Immunol. **165**:1395.
28. Flier, J. *et al.* (1999) J. Invest. Dermatol. **113**:574.
29. Sauty, A. *et al.* (1999) J. Immunol. **162**:3549.
30. Brice, G.T. *et al.* (2001) J. Immunol. Methods **194**:1375.
31. Marx, N. *et al.* (2000) J. Immunol. **164**:6503.
32. Sheikh, A.M. *et al.* (2001) Ann. N.Y. Acad. Sci. **947**:306.
33. Kakimi, K. *et al.* (2001) J. Exp. Med. **194**:1755.
34. Sgadari, C. *et al.* (1997) Blood **89**:2635.
35. Mahalingham, S. *et al.* (1999) J. Virol. **73**:1479.

REFERENCES *CONTINUED*

36. Liu, M.T. *et al.* (2001) J. Immunol. **166**:1790.
37. Palmer, K. *et al.* (2001) Gene Ther. **8**:282.
38. Salazar-Mather, T.P. *et al.* (2000) J. Clin. Invest. **105**:985.
39. Pulaski, B.A. *et al.* (2000) Cancer Immunol. Immunother. **49**:34.
40. Liu, M.T. *et al.* (2001) Adv. Exp. Med. Biol. **494**:323.
41. Ruehlmann, J.M. *et al.* (2001) Cancer Res. **61**:8498.
42. Melter, M. *et al.* (2001) Circulation **104**:2558.
43. Kapoor, A. *et al.* (2000) Transplantation **69**:1147.
44. Hancock, W.W. *et al.* (2000) J. Exp. Med. **192**:1515.
45. Yun, J.J. *et al.* (2001) J. Heart Lung. Transplant **20**:156.
46. Koga, S. *et al.* (2001) Transplant. Proc. **33**:549.
47. Koga, S. *et al.* (2001) Transplant. Proc. **33**:459.
48. Hancock, W.W. *et al.* (2001) J. Exp. Med. **193**:975.
49. Halloran, P.F. *et al.* (2001) J. Immunol. **166**:7072.
50. Meyer, M. *et al.* (2001) Eur. J. Immunol. **31**:2521.
51. Simpson, J.E. *et al.* (2000) Neuropathol. Appl. Neurobiol. **26**:133.
52. Mach, F. *et al.* (1999) J. Clin. Invest. **104**:1041.
53. Goebeler, M. *et al.* (1998) J. Pathol. **184**:89.
54. Patel, D.D. *et al.* (2001) Clin. Immunol. **98**:39.
55. Koga, S. *et al.* (1999) J. Immunol. **163**:4878.
56. Miura, M. *et al.* (2001) J. Immunol. **167**:3494.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES