Quantikine® ELISA

Human CCL18/PARC Immunoassay

Catalog Number DCL180B

For the quantitative determination of human Chemokine C-C motif Ligand 18 (CCL18) concentrations in cell culture supernates, tissue lysates, serum, plasma, and urine.

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

Human CCL18 (Chemokine CC Motif Ligand 18), also known as PARC (Pulmonary and Activation-Regulated Chemokine), alternative macrophage activation-associated CC chemokine 1 (AMAC-1), macrophage inflammatory protein-4 (MIP-4), and dendritic cell-derived chemokine 1 (DCCK1), belongs to the Cys-Cys (CC) motif-containing chemokine family. It is an 89 amino acid (aa) residue precursor protein with a 20 aa putative signal peptide that is cleaved to generate a 69 aa residue mature protein (1, 2). CCL18 is highly expressed in lung, lymph nodes and placenta. It was initially identified as a chemotactic factor for naive $CD4^+$ T cells and immature dendritic cells, suggesting that it plays an important role in immune responses (3, 4). It displays strong chemotactic activity for naive $CD4^+$ and $CD8^+$ T cells and nonactivated lymphocytes. It attracts naive T lymphocytes toward dendritic cells and activated macrophages in lymph nodes. CCL18 is mainly induced by Th2 type cytokines, such as IL-4 and IL-13, and inhibited by IFN-γ (2). CCL18 can also facilitate the generation of tolerogenic dendritic cells and adaptive regulatory T cells (5, 6). Besides its chemotactic effects, CCL18 enhances the proliferation of monocytes and increases cytokine production (7). Additionally, CCL18 is involved in stimulation of collagen production by fibroblasts. Thus, it may directly contribute to the development of pulmonary fibrosis by stimulating collagen production (8, 9). CCL18 is an orphan chemokine without any known receptor. A rodent homolog has not been identified (10). High levels of CCL18 have been detected in various body fluids, such as serum and synovial fluid (11).

CCL18 is dysregulated in a number of pathological conditions. Increased CCL18 in bronchoalveolar lavages and sera has been found to be linked to allergic asthma (12). CCL18 is able to mediate skin homing of human memory T cells. It was present in skin biopsies of atopic dermatitis patients but not in normal skin (13). It has been reported that CCL18 serum levels correlate with the disease severity of atopic dermatitis (14). Elevated CCL18 levels were detected in synovial fluid from rheumatoid arthritis patients, suggesting that it may be involved in the autoimmune and inflammatory process (15). Additionally, elevated serum CCL18 reflects lung fibrosis activity in systemic sclerosis and could be an early marker of lung function worsening (16). In patients with chronic obstructive pulmonary diseases, serum CCL18 levels are also elevated and may be useful to track clinical outcomes (17). Furthermore, investigations indicate that CCL18 is a potential valuable diagnostic and prognostic biomarker in coronary artery disease (18, 19).

The Quantikine Human CCL18/PARC Immunoassay is a 2.5 hour solid-phase ELISA designed to measure human CCL18 in cell culture supernates, tissue lysates, serum, plasma, and urine. It contains *E. coli*-expressed recombinant human CCL18 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CCL18 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 CCL18.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human CCL18 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CCL18 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human CCL18 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 CCL18 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.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human CCL18/PARC Microplate	894227	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human CCL18.	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 CCL18/PARC Standard	894862	2 vials of recombinant human CCL18 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a fresh standard for each assay.
Human CCL18/PARC Conjugate	894861	21 mL of a polyclonal antibody specific for human CCL18 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 buffer with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:5 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservatives. *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.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human CCL18 Controls (optional; R&D Systems, Catalog # QC59B).

SUPPLIES REQUIRED FOR TISSUE LYSATE SAMPLES

- PBS
- IC Diluent # 4 (R&D Systems, Catalog # DYC001).
  or
- RIPA Buffer

PRECAUTIONS

CCL18 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® 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

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.

Tissue Lysates - Cells must be lysed prior to assay as directed in the Tissue Lysis Procedure.

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 heparin or EDTA 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.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

TISSUE LYSIS PROCEDURE

Use the following procedure for the preparation of tissue lysate samples.

1. Homogenize tissue for 2 minutes in cold PBS.
2. Centrifuge at 12,000 x g for 30 minutes to remove supernatant.
3. Cover the cell pellet with IC Diluent # 4 and briefly mix in a microgrinder at 6500 rpm.
4. Centrifuge at 12,000 x g for 30 minutes to remove cell debris.
5. Assay immediately or aliquot the lysis supernates and store at ≤ -70 °C until ready for use.

SAMPLE PREPARATION

Cell culture supernate and urine samples require a 2-fold dilution due to matrix effects. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD5P (diluted 1:5).*

Tissue lysate samples require a 50-fold dilution due to matrix effects. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5P (diluted 1:5).

Serum and plasma samples require a 100-fold dilution due to high endogenous levels. A suggested 100-fold dilution is 10 µL of sample + 990 µL of Calibrator Diluent RD5P (diluted 1:5).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *CCL18 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

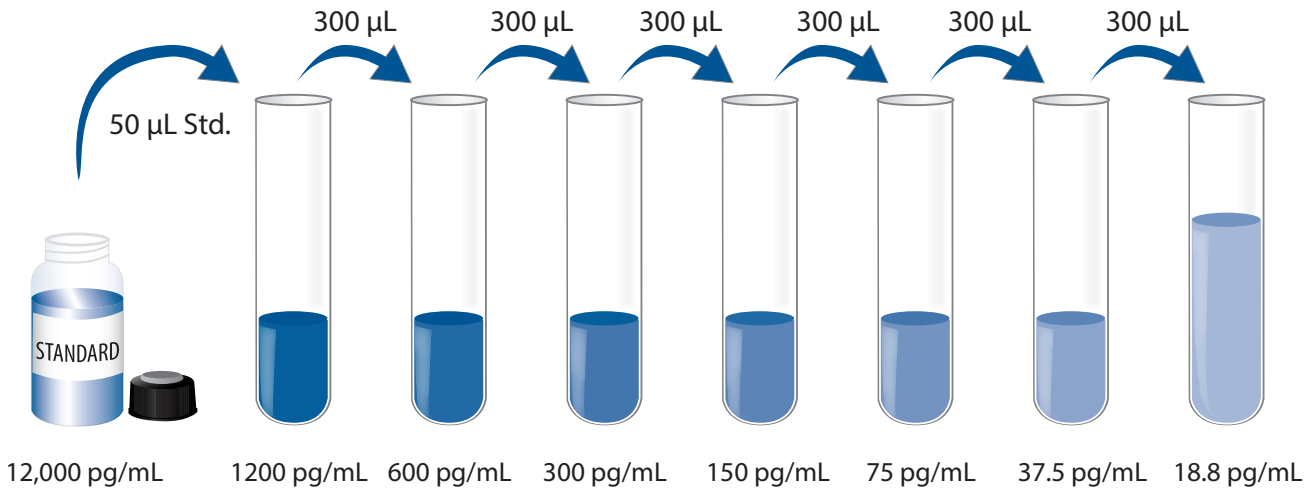
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human CCL18/PARC Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human CCL18/PARC Standard with deionized or distilled water. This reconstitution produces a stock solution of 12,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes. Mix well prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5P (diluted 1:5) into the 1200 pg/mL tube. Pipette 300 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 1200 pg/mL standard serves as the high standard. Calibrator Diluent RD5P (diluted 1:5) 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.

Note: *CCL18 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 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 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human CCL18/PARC 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 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

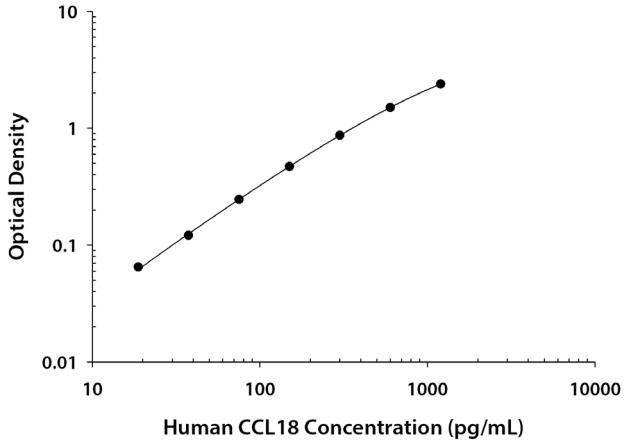
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a 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 human CCL18 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.

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

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.024	0.023	—
18.8	0.087 0.088	0.088	0.065
37.5	0.142 0.146	0.144	0.121
75.0	0.265 0.273	0.269	0.246
150	0.492 0.496	0.494	0.471
300	0.891 0.895	0.893	0.870
600	1.519 1.535	1.527	1.504
1200	2.383 2.447	2.415	2.392

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 twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	70.8	248	592	84.2	265	614
Standard deviation	2.27	8.21	21.8	5.10	17.1	27.6
CV (%)	3.2	3.3	3.7	6.1	6.5	4.5

RECOVERY

The recovery of human CCL18 spiked to levels throughout the range of the assay in various matrices was evaluated. Samples were diluted prior to assay.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	91-110%
Tissue lysis buffer (n=1)	92	91-93%
Urine (n=4)	104	92-112%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human CCL18 were serially diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Extraction buffer (n=1)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)
1:2	Average % of Expected	100	109	98	100	100	98
	Range (%)	98-102	———	96-99	95-105	96-104	94-100
1:4	Average % of Expected	98	112	97	97	102	98
	Range (%)	94-100	———	94-98	93-102	95-107	92-102
1:8	Average % of Expected	99	118	97	97	100	96
	Range (%)	97-101	———	94-102	92-102	93-106	90-101
1:16	Average % of Expected	101	114	97	98	103	95
	Range (%)	100-101	———	96-100	93-102	93-112	92-99

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human CCL18 ranged from 0.554-1.77 pg/mL. The mean MDD was 1.12 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.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human CCL18 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human CCL18 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=36)	55,646	21,243-104,556	21,409
EDTA plasma (n=36)	52,494	18,927-106,291	22,160
Heparin plasma (n=36)	56,025	20,294-105,663	23,560

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=10)	67.6	40	ND-91.9

ND=Non-detectable

Cell Culture Supernates:
Human peripheral blood mononuclear cells (50 x 106 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate overnight. Cells were unstimulated or stimulated with 1 µg/mL LPS for 24 hours. Aliquots of the cell culture supernates were removed, assayed for human CCL18, and measured 962 pg/mL and 2132 pg/mL, respectively.

Human monocyte-derived macrophages were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate overnight. Cells were unstimulated or stimulated with 1 µg/mL LPS for 24 hours. Aliquots of the cell culture supernates were removed, assayed for human CCL18, and measured 389 pg/mL and 504 pg/mL, respectively.

Human dendritic cells were isolated from peripheral blood using a cell enrichment kit and cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, 50 ng/mL of recombinant human GM-CSF, and 25 ng/mL recombinant human IL-4 for 6 days. An aliquot of the cell culture supernate was removed, assayed for human CCL18, and measured 58.2 pg/mL.

Tissue Lysates - Human placentas were lysed according to the Tissue Lysis Procedure. An aliquot of the lysate was removed, assayed for human CCL18, and measured 3517 pg/mL.

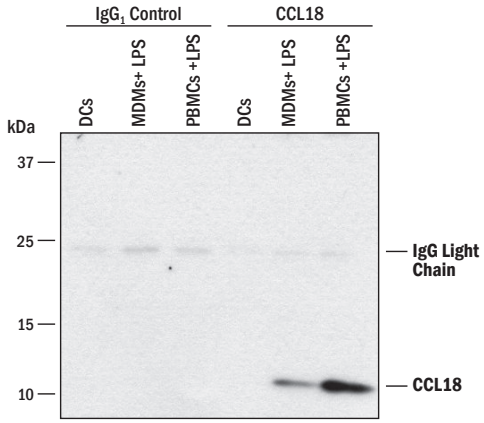
SPECIFICITY

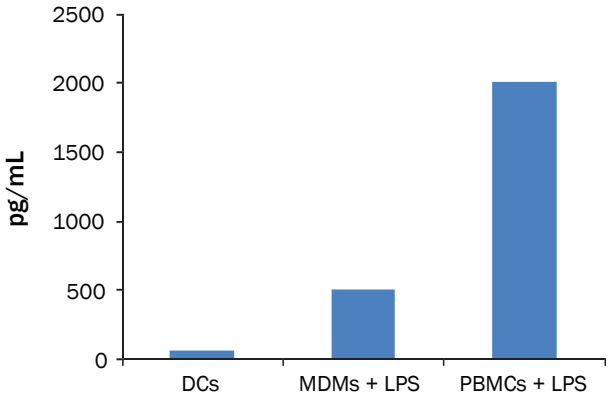
This assay recognizes natural and recombinant human CCL18.

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 CCL18 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

MIP-1α
MIP-1β
RANTES





Conditioned media samples from human dendritic cells (DCs), monocyte-derived macrophages (MDMs), or peripheral blood mononuclear cells (PBMCs) were analyzed by Immunoprecipitation/Western blot and Quantikine ELISA. For immunoprecipitation, the capture antibody in this kit and 50 µL of Protein G Sepharose beads were incubated overnight. Immunoprecipitated samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody in this kit. The Immunoprecipitation/Western blot shows a direct correlation with the ELISA value for these samples.

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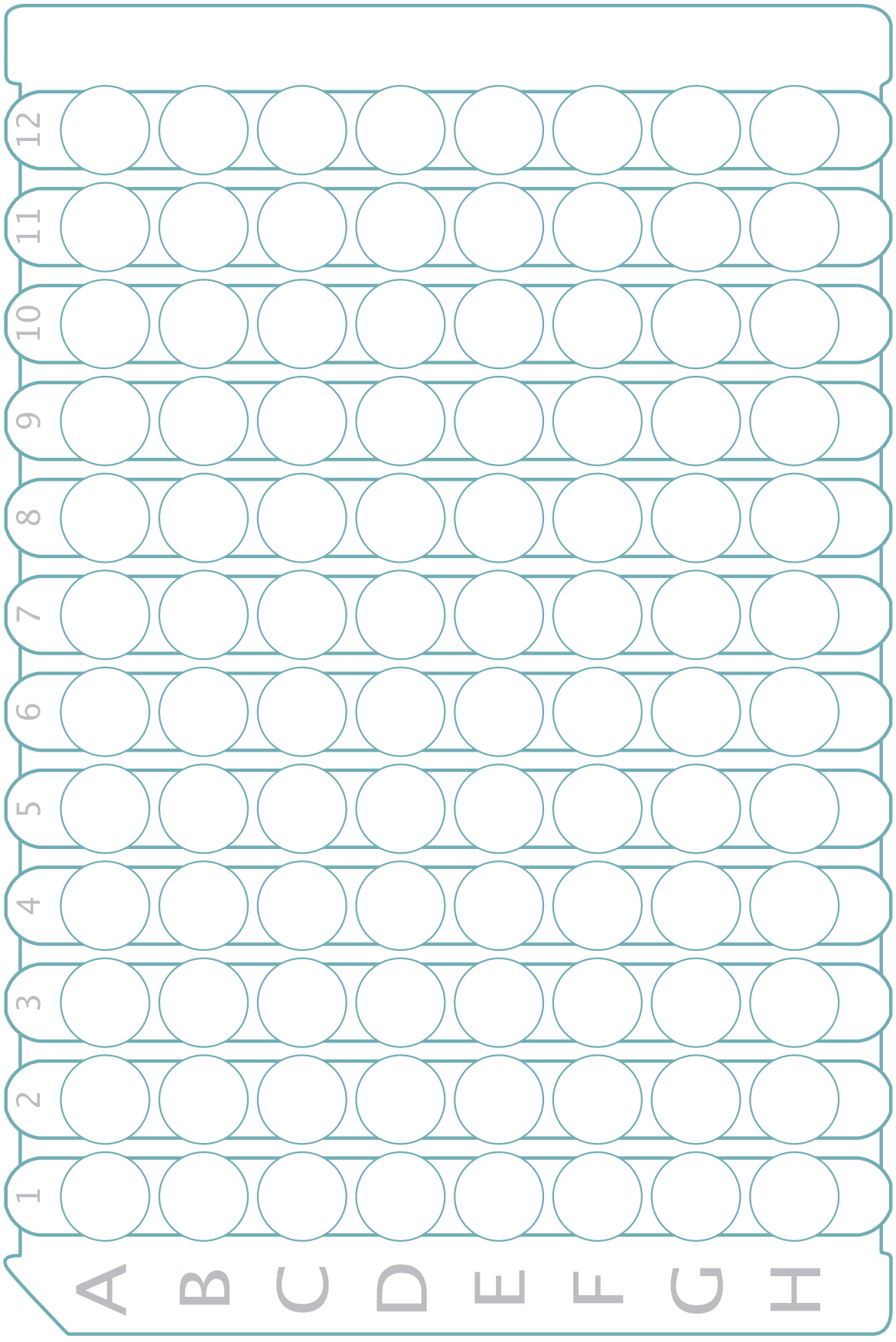
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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