

# Quantikine<sup>®</sup>

## Mouse CXCL1/KC Immunoassay

Catalog Number MKC00B

SMKC00B

PMKC00B

**For the quantitative determination of mouse KC concentrations in cell culture supernates and mouse serum.**

*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

Mouse KC, also known as CXCL1 or N51, was originally identified in fibroblasts as a PDGF-induced immediate early gene that encodes a secretory protein of approximately 8 kDa (1 - 3). The protein sequence of mouse KC identified it as a member of the alpha (CXC) chemokine family of inflammatory and immunoregulatory cytokines (4). Besides mitogen-stimulated fibroblasts, KC expression can be induced in bacterial or LPS-stimulated peritoneal and lung macrophages, endothelial cells and vascular smooth cells (5). The induction of KC by mitogens has been shown to be inhibited by glucocorticoids (6).

Mouse KC cDNA encodes a 96 amino acid residue precursor protein from which the amino-terminal 19 amino acid residues are cleaved to generate the 77 amino acid residue mature KC (2). The protein sequence of mouse KC shows approximately 63% identity to that of mouse MIP-2, another mouse alpha chemokine. In addition, the protein sequence of KC is approximately 60% identical to the human GROs (2). Like other alpha chemokines, mouse KC is a potent neutrophil attractant and activator. The activities of KC and MIP-2 have been shown to be mediated by the unique mouse IL-8 receptor that shows 71% and 68% amino acid sequence identity to human IL-8R $\beta$  and IL-8R $\alpha$ , respectively (7, 8). Since an IL-8 homolog has not been identified in mice, it has been suggested that MIP-2 and KC are the functional homologs of IL-8 and may function as the major proinflammatory alpha chemokines in mice. Increased KC expression has been found to be associated with neutrophil influx in various inflammatory conditions (5, 9 - 11).

The Quantikine Mouse KC Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse KC in cell culture supernates and mouse serum. It contains *E. coli*-expressed recombinant mouse KC and antibodies raised against the recombinant factor. This Immunoassay has been shown to quantitate the recombinant mouse KC accurately. Results obtained using natural mouse KC showed dose-response curves that were parallel to the standard curves obtained using the recombinant kit standards. These results indicate that the Quantikine Mouse KC Immunoassay kit can be used to determine relative mass values for natural mouse KC.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An affinity purified polyclonal antibody specific for mouse KC has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any mouse KC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse KC is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of mouse KC bound in the initial step. The sample values are then read off the standard curve.

## 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 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 binding proteins, receptors, and other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

## PRECAUTION

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

## 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

## MATERIALS PROVIDED

Description	Part #	Cat. # MKC00B	Cat. # SMKC00B
<b>Mouse KC Microplates</b> - 96 well polystyrene microplates (12 strips of 8 wells) coated with polyclonal antibody specific for mouse KC.	890787	2 plates	6 plates
<b>Mouse KC Conjugate</b> - 23 mL/vial of a polyclonal antibody against mouse KC conjugated to horseradish peroxidase with preservatives.	892703	1 vial	3 vials
<b>Mouse KC Standard</b> - 5 ng/vial of recombinant mouse KC in a buffered protein base with preservatives; lyophilized.	890786	1 vial	3 vials
<b>Mouse KC Control</b> - Recombinant mouse KC in a buffered protein base with preservatives; lyophilized. The concentration range of mouse KC after reconstitution is shown on the vial label. The assay value of the Control should be within the range specified on the label.	890789	1 vial	3 vials
<b>Assay Diluent RD1-18</b> - 12.5 mL/vial of a buffered protein solution with preservatives.	895202	1 vial	3 vials
<b>Calibrator Diluent RD5-3</b> - 21 mL/vial of a buffered protein solution with preservatives.	895436	2 vials	6 vials
<b>Wash Buffer Concentrate</b> - 50 mL/vial of a 25-fold concentrated solution of a buffered surfactant with preservative.	895024	1 vial	3 vials
<b>Color Reagent A</b> - 12 mL/vial of stabilized hydrogen peroxide.	895000	1 vial	3 vials
<b>Color Reagent B</b> - 12 mL/vial of stabilized chromogen (tetramethylbenzidine).	895001	1 vial	3 vials
<b>Stop Solution</b> - 23 mL/vial of a diluted hydrochloric acid solution.	895174	1 vial	3 vials
<b>Plate Covers</b> - Adhesive strips.	640197	8 strips	24 strips

MKC00B contains sufficient materials to run ELISAs on two 96 well plates.

SMKC00B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PMKC00B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

## STORAGE

<b>Unopened Kit</b>	Store at 2 - 8° C. Do not use beyond kit expiration date.	
<b>Opened/ Reconstituted Reagents</b>	Mouse KC Conjugate	May be stored for up to 1 month at 2 - 8° C.*
	Diluted Wash Buffer	
	Stop Solution	
	Calibrator Diluent RD5-3	
	Assay Diluent RD1-18	
	Unmixed Color Reagent A	
	Unmixed Color Reagent B	Aliquot and store for up to 1 month at ≤ -20° C in a manual defrost freezer.*
	Mouse KC Standard (1000 pg/mL)	
	Mouse KC Control	
	Microplate Wells	Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8° C.*

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

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 1000 mL graduated cylinders.

## SAMPLE COLLECTION AND STORAGE

**Cell Culture Supernates** - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

**Serum** - Allow blood samples to clot for 2 hours at room temperature or overnight at 2 - 8° C before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

**Note:** *Grossly hemolyzed or lipemic samples may not be suitable for measurement of mouse KC with this assay.*

## SAMPLE PREPARATION

Mouse serum samples require a 2-fold dilution into Calibrator Diluent RD5-3 prior to assay. A suggested dilution is 70  $\mu\text{L}$  sample + 70  $\mu\text{L}$  Calibrator Diluent RD5-3. Mix well.

## REAGENT PREPARATION

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

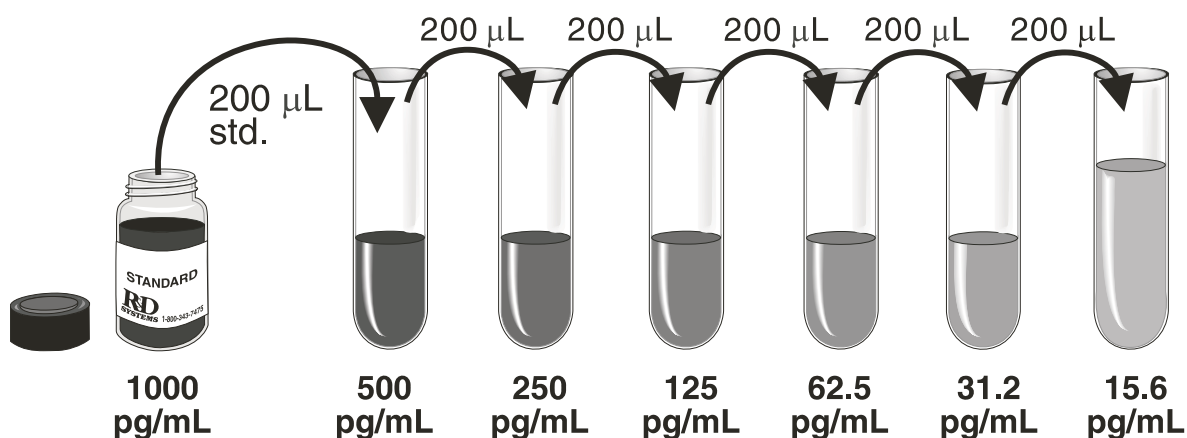
**Mouse KC Kit Control** - Reconstitute the Kit Control with 1.0 mL deionized or distilled water. Assay the Control undiluted.

**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 25 mL Wash Buffer Concentrate into deionized or distilled water to prepare 625 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. 100  $\mu\text{L}$  of the resultant mixture is required per well.

**Mouse KC Standard** - Reconstitute the mouse KC Standard with 5.0 mL of Calibrator Diluent RD5-3. Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200  $\mu\text{L}$  of Calibrator Diluent RD5-3 into each tube. Use the standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted mouse KC Standard serves as the high standard (1000 pg/mL). Calibrator Diluent RD5-3 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, standards, and control be assayed in duplicate.**

1. Prepare all reagents, standard dilutions, control, 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  $\mu\text{L}$  of Assay Diluent RD1-18 to each well.
4. Add 50  $\mu\text{L}$  of Standard, Control, or sample\* per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. Plate layouts are provided to record the standards and samples assayed.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400  $\mu\text{L}$ ) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100  $\mu\text{L}$  of Mouse KC 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 100  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100  $\mu\text{L}$  of Stop Solution to each well. 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.

\*Mouse serum samples require a 2-fold dilution into Calibrator Diluent RD5-3, as directed in the Sample Preparation section.

## PROCEDURE SUMMARY AND CHECKLIST

1.  Bring all reagents to room temperature.  
 Prepare reagents and samples as instructed.  
 Return unused components to storage temperature as indicated in the instructions.
2.  Add 50  $\mu$ L Assay Diluent to each well.
3.  Add 50  $\mu$ L Standard, Control, or sample\* to each well.  
 Tap plate gently for one minute.  
 Cover the plate and incubate for 2 hours at room temperature.
4.  Aspirate and wash each well five times.
5.  Add 100  $\mu$ L Conjugate to each well.  
 Cover the plate and incubate for 2 hours at room temperature.
6.  Aspirate and wash each well five times.
7.  Add 100  $\mu$ L Substrate Solution to each well. Incubate 30 minutes at room temperature. **Protect from light.**
8.  Add 100  $\mu$ L Stop Solution to each well.
9.  Read Optical Density at 450 nm (correction wavelength set at 540 nm or 570 nm).

\*Serum samples require dilution. See Sample Preparation section.

## CALCULATION OF RESULTS

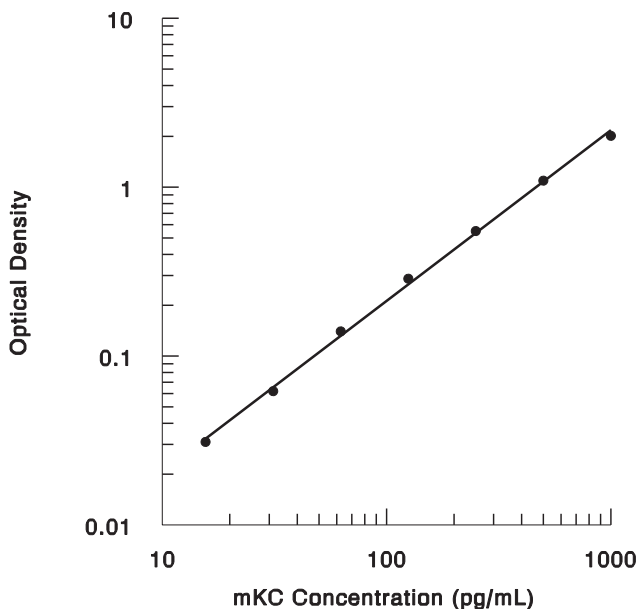
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density.

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the mouse KC concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

Because serum samples have been diluted prior to the assay, the measured concentrations 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	0.083 0.079 0.112	0.081	—
15.6	0.112 0.149	0.112	0.031
31.2	0.156 0.220	0.152	0.071
62.5	0.231 0.345	0.226	0.145
125	0.359 0.601	0.352	0.271
250	0.659 1.118	0.630	0.549
500	1.156 2.031	1.137	1.056
1000	2.060	2.046	1.965

## PRECISION

### Intra-assay Precision (Precision within an assay)

Three samples of known concentration were tested twenty times 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.

Sample	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	34.9	169	530	35.0	183	521
Standard deviation	1.9	5.2	26.3	2.1	18.0	15.6
CV (%)	5.4	3.1	5.0	6.0	9.8	3.0

## RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates (n = 7)	98	89 - 103%
Mouse serum* (n = 9)	95	83 - 104%

\*Mouse serum samples were first diluted 2-fold as directed in the Sample Preparation section.

## LINEARITY

To assess the linearity of the assay, five or more samples containing and/or spiked with various concentrations of mouse KC in each matrix were diluted with Calibrator Diluent RD5-3 and then assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell Culture Supernates	neat	662		
	1:2	316	331	95
	1:4	150	166	90
	1:8	79	83	95
	1:16	38	42	90
Mouse Serum*	spiked	627		
	1:2	315	314	100
	1:4	165	157	105
	1:8	80	78	103
	1:16	42	39	108

\*Mouse serum samples were first diluted 2-fold, as directed in the Sample Preparation section.

## SENSITIVITY

The minimum detectable dose of mouse KC is typically less than 2.0 pg/mL.

The minimum detectable dose was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

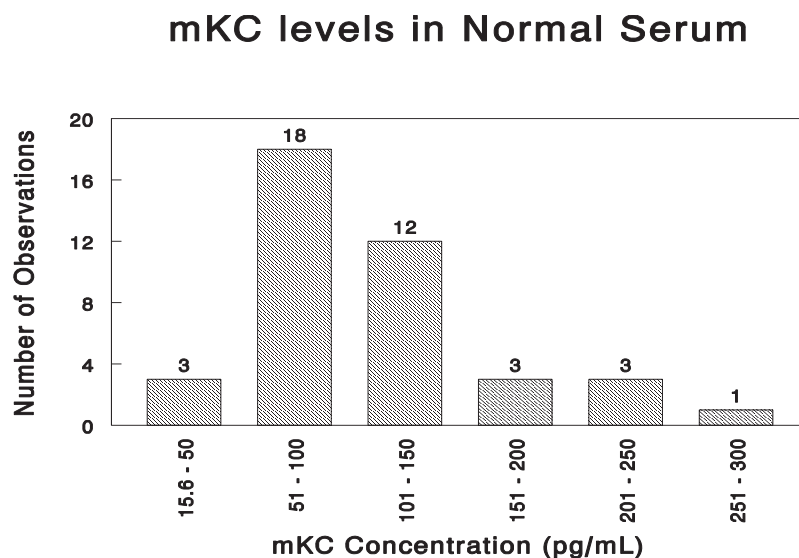
## CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant mouse KC produced at R&D Systems. This recombinant mouse KC contains 77 amino acid residues and has a predicted molecular mass of 8 kDa.

The protein concentration of the recombinant mouse KC was determined by the method of Bradford (12) using purified bovine serum albumin as a standard.

## SAMPLE VALUES

**Serum** - Forty individual mouse serum samples were evaluated for detectable levels of mouse KC in this assay. The mean mouse KC value and standard deviation were  $109 \pm 54$  pg/mL. Measured mouse KC concentrations are reported in the following histogram.



**Cell Culture Supernates** - Mouse heart conditioned media (1 heart, 1 - 2 mm pieces in 10 mL of RPMI supplemented with 10% fetal bovine serum) were collected after culturing for 5 days. The culture supernate was assayed for mouse KC and measured 20 ng/mL.

## SPECIFICITY

This assay recognizes both recombinant and natural mouse KC. The factors listed below were prepared at 50 ng/mL in Calibrator Diluent RD5-3 and assayed for cross-reactivity.

Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse KC control were assayed for interference. No significant cross-reactivity or interference was observed.

### Recombinant mouse:

C10	IL-5
G-CSF	IL-6
GM-CSF	IL-7
IFN- $\gamma$	IL-9
IL-1 $\alpha$	IL-10
IL-1 $\beta$	IL-10 sR
IL-2	IL-12
IL-3	IL-13
IL-4	JE/MCP-1

LIF
M-CSF
MIP-1 $\alpha$
MIP-1 $\beta$
MIP-2
SCF
TNF- $\alpha$
Tpo
VEGF

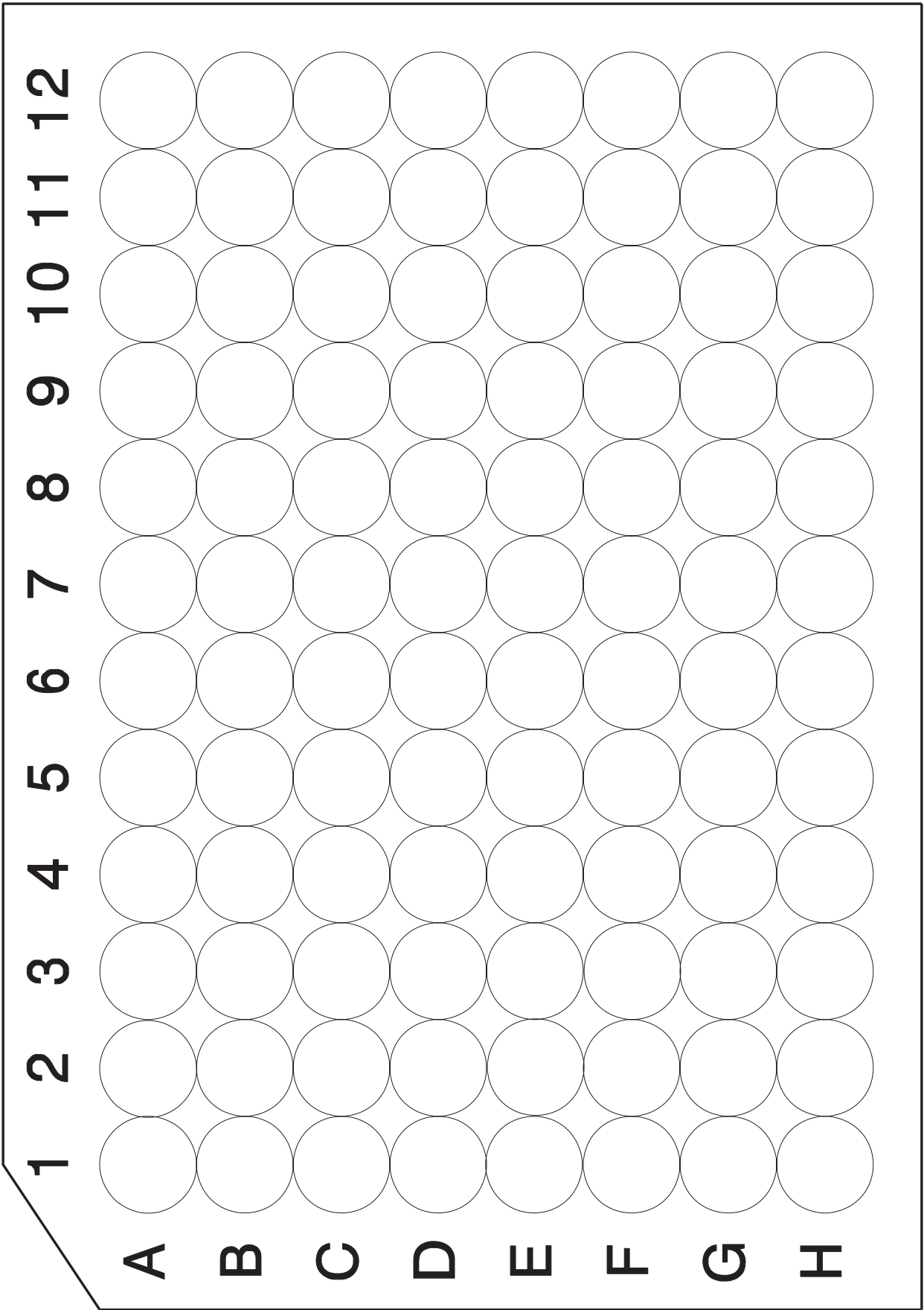
### Recombinant human:

GRO $\alpha$
GRO $\beta$
IL-8
MIP-1 $\alpha$
MIP-1 $\beta$
RANTES

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