Quantikine™ ELISA

Mouse CXCL1/KC Immunoassay

Catalog Number MKC00B-1
SMKC00B
PMKC00B

For the quantitative determination of mouse Keratinocyte-derived Cytokine (KC) concentrations in cell culture supernates and 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 (aa) residue precursor protein from which the amino-terminal 19 aa residues are cleaved to generate the 77 aa 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% aa sequence identity to human IL-8 Rβ and IL-8 Rα, respectively (7, 8). Since an IL-8 homolog has not been identified in mice, its 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 CXCL1/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 Quantikine kit standards. These results indicate that this 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. A polyclonal antibody specific for mouse KC has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any 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 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.
- 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.
- For best results, pipette reagents and samples into the center of each well.
- 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.

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. Refer to the SDS 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 #	CATALOG # MKC00B-1	CATALOG # SMKC00B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse KC Microplate	890787	1 plate	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with a polyclonal antibody specific for mouse KC.	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.*
Mouse KC Standard	890786	1 vial	3 vials	Recombinant mouse KC in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.*
Mouse KC Control	890789	1 vial	3 vials	Recombinant mouse KC in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse KC Conjugate	899528	1 vial	6 vials	12.5 mL/vial of a polyclonal antibody specific for mouse KC conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-18	895202	1 vial	3 vials	12 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-3	895436	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of a buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

MKC00B-1 contains sufficient materials to run ELISAs on one 96 well plate.
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). 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.
  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
Mouse KC Microplate	890787	50 plates
Mouse KC Standard*	890786	25 vials
Mouse KC Control	890789	25 vials
Mouse KC Conjugate	899528	50 vials
Assay Diluent RD1-18	895202	25 vials
Calibrator Diluent RD5-3	895436	50 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Stop Solution	895174	25 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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

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 - Allow blood samples to clot for 2 hours at room temperature 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 are not suitable for use in this assay.*

SAMPLE PREPARATION

Serum samples require a 2-fold dilution prior to assay. A suggested dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-3.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

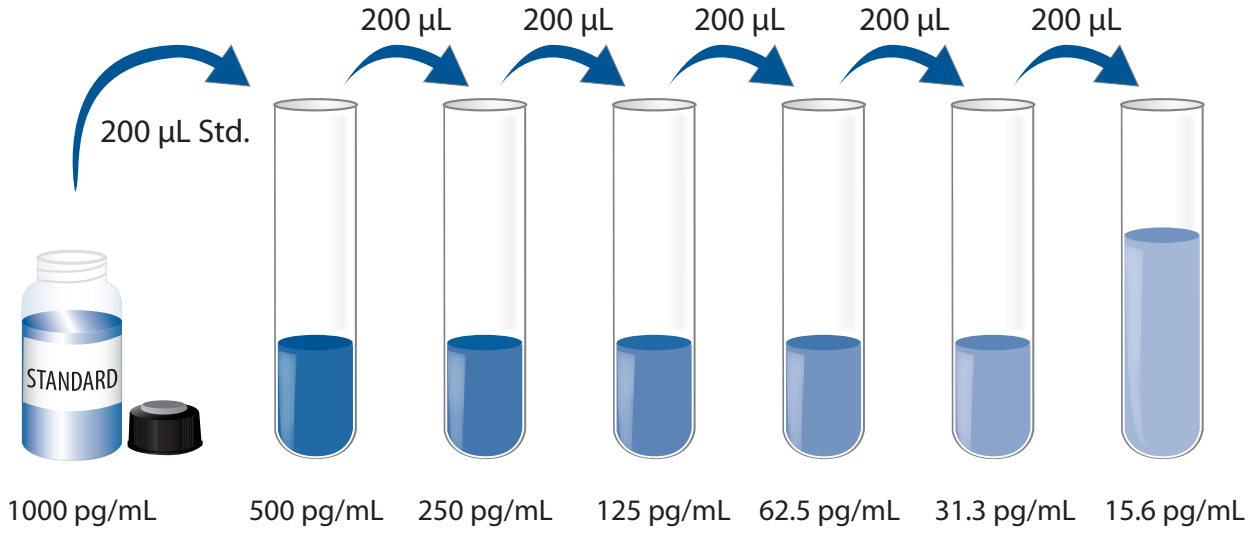
Mouse KC Control - Reconstitute the control with 1 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate into 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. 100 µL of the resultant mixture is required per well.

Mouse KC Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse KC Standard with 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 µL of Calibrator Diluent RD5-3 into each tube. Use the standard stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse KC Standard (1000 pg/mL) serves as the high standard. 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 standards, control, and samples 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 µL of Assay Diluent RD1-18 to each well.
4. Add 50 µL 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.
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 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 100 µL 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 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

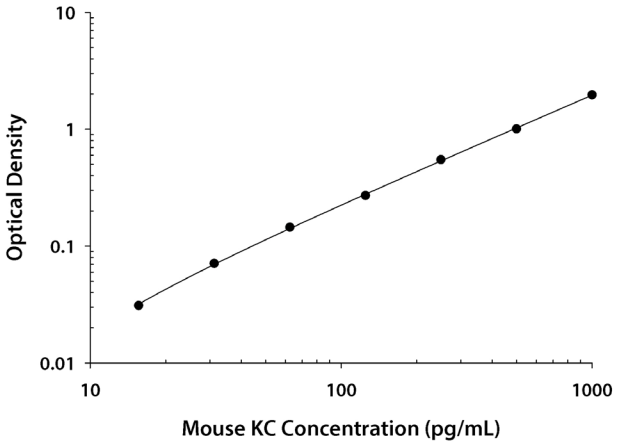
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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

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	0.083 0.079	0.081	—
15.6	0.112 0.112	0.112	0.031
31.3	0.149 0.156	0.152	0.071
62.5	0.220 0.231	0.226	0.145
125	0.345 0.359	0.352	0.271
250	0.601 0.659	0.630	0.549
500	1.118 1.156	1.137	1.056
1000	2.031 2.060	2.046	1.965

SENSITIVITY

The minimum detectable dose (MDD) of mouse KC is typically less than 2.0 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.

CALIBRATION

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

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)	34.9	169	530	35.0	183	521
Standard deviation	1.90	5.20	26.3	2.10	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%
Serum* (n=9)	95	83-104%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of mouse KC in each matrix were diluted with calibrator diluent and then assayed.

		Cell culture supernates (n=4)	Serum* (n=4)
1:2	Average % of Expected	103	100
	Range (%)	96-109	91-108
1:4	Average % of Expected	102	99
	Range (%)	95-112	95-107
1:8	Average % of Expected	102	103
	Range (%)	88-111	94-108
1:16	Average % of Expected	101	100
	Range (%)	87-110	91-109

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SAMPLE VALUES

Serum - Samples were evaluated for detectable levels of mouse KC in this assay.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=32)	114	41-255	58

Cell Culture Supernates - Mouse heart (1 heart, 1-2 mm pieces) was cultured in RPMI supplemented with 10% fetal bovine serum for 5 days. The cell culture supernate was removed, assayed for mouse KC, and measured 20 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse KC.

The factors listed below were prepared at 50 ng/mL in calibrator diluent 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
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-10 R
IL-12
IL-5
IL-6
IL-7
IL-9
IL-10
IL-13
JE/MCP-1
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
SCF
TNF-α
Tpo
VEGF

Recombinant human:

GROα
GROβ
IL-8
MIP-1α
MIP-1β
RANTES

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