Quantikine® ELISA

Rat CXCL2/CINC-3 Immunoassay

Catalog Number RCN300

For the quantitative determination of rat Cytokine-Induced Neutrophil Chemoattractant-3 (CINC-3) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

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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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Rat CINC (Cytokine-Induced Neutrophil Chemoattractant) proteins, including CINC-1, -2α, -2β, and -3, are orthologs of the human GRO (α, β, and γ) proteins (1). These proteins contain the ELRCXC motif and are members of the CXC chemokine family (1-6). All CINC proteins exist as approximately 13-14 kDa non-covalently-linked homodimers in solution (1, 7, 9). They signal via the chemokine receptor CXCR2 to induce neutrophil chemotaxis and calcium mobilization (1-6). CINC-3, also known as MIP-2 (Macrophage Inflammatory Protein 2), is synthesized as a 100 amino acid (aa) residue precursor with a 31 aa signal peptide and a 69 aa mature segment (1, 8-10). It shares 67% aa sequence identity with CINC-1 (1, 11). CINC-3 also shares approximately 78% aa sequence identity with CINC-2α and -2β, which are products of alternative splicing that differ only in their C-terminal three aa residues (1, 12). Cells known to express CINC-3 include neutrophils (13, 14), fibroblasts (15), endothelial cells (15), mast cells (16), intestinal epithelium (17), and macrophages (18).

The Quantikine® Rat CXCL2/CINC-3 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure rat CINC-3 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant rat CINC-3 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural rat CINC-3 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 natural rat CINC-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for rat CINC-3 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any CINC-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat CINC-3 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 CINC-3 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, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in 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.
- 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 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
Rat CINC-3 Microplate	892990	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for rat CINC-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.*
Rat CINC-3 Conjugate	892991	12 mL of a polyclonal antibody specific for rat CINC-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Rat CINC-3 Standard	892992	Recombinant rat CINC-3 in a buffered protein base, with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Rat CINC-3 Control	892993	Recombinant rat CINC-3 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-33	895349	21 mL of diluted animal serum with preservatives.	
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	895174	23 mL of diluted hydrochloric 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.
- 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. 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 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 20 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.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

SAMPLE PREPARATION

Cell culture supernate samples require at least a 4-fold dilution prior to assay. A suggested 4-fold dilution is 30 µL of sample + 90 µL of Calibrator Diluent RD6-33.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

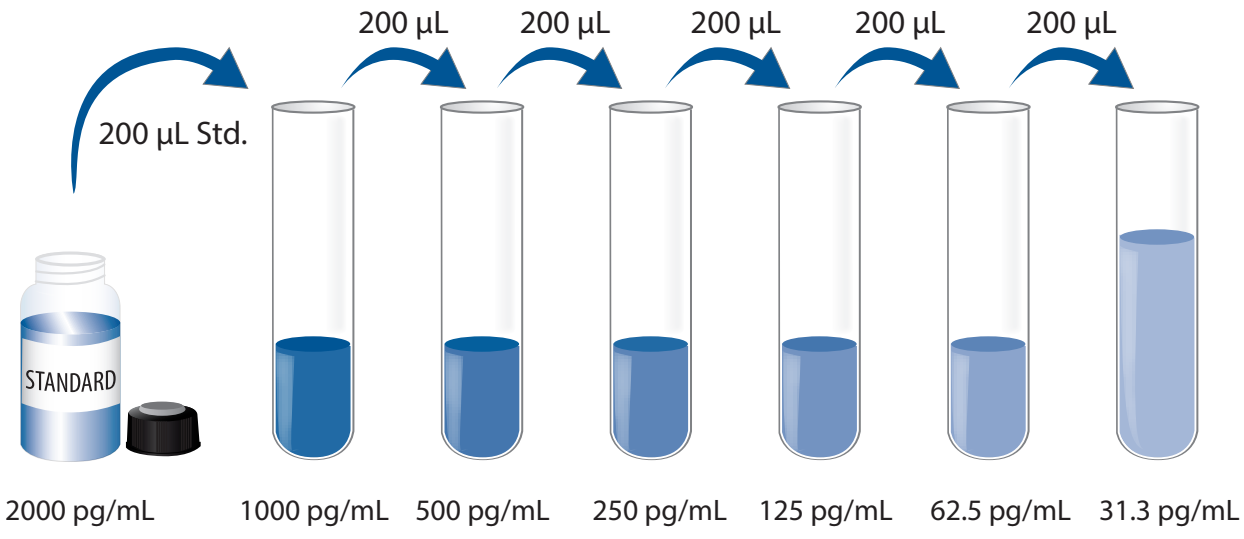
Rat CINC-3 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

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 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. 100 µL of the resultant mixture is required per well.

Rat CINC-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat CINC-3 Standard with Calibrator Diluent RD6-33. Do not substitute other diluents. This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD6-33 into each tube. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Rat CINC-3 Standard (2000 pg/mL) serves as the high standard. Calibrator Diluent RD6-33 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 RD1-21 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 Rat CINC-3 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

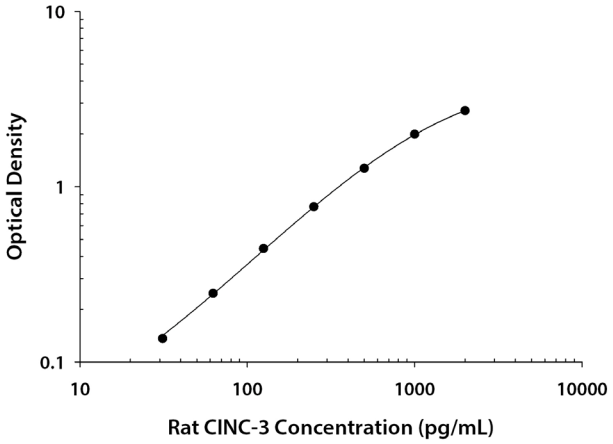
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 rat CINC-3 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	0.008 0.008	0.008	—
31.3	0.141 0.147	0.144	0.136
62.5	0.253 0.254	0.254	0.246
125	0.446 0.455	0.451	0.443
250	0.772 0.779	0.776	0.768
500	1.267 1.294	1.281	1.273
1000	1.979 2.013	1.996	1.988
2000	2.712 2.724	2.718	2.710

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 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	50	46	50
Mean (pg/mL)	111	345	1012	105	308	877
Standard deviation	3.9	11.9	39.1	10.1	28.5	73.2
CV (%)	3.5	3.4	3.9	9.6	9.3	8.3

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture supernates* (n=6)	97	86-107%
Serum (n=6)	97	83-106%
EDTA plasma (n=6)	92	86-99%
Heparin plasma (n=6)	99	85-105%

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

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of rat CINC-3 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates* (n=12)	Serum (n=6)	EDTA plasma (n=6)	Heparin plasma (n=6)
1:2	Average % of Expected	98	102	104	102
	Range (%)	86-110	97-111	100-109	94-107
1:4	Average % of Expected	95	101	103	102
	Range (%)	83-109	96-114	99-108	95-108
1:8	Average % of Expected	99	102	103	101
	Range (%)	81-114	89-116	98-109	94-107
1:16	Average % of Expected	101	107	104	103
	Range (%)	89-116	102-117	93-113	97-110

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

SENSITIVITY

Forty-three assays were evaluated and the minimum detectable dose (MDD) of rat CINC-3 ranged from 0.5-2.7 pg/mL. The mean MDD was 1.0 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 rat CINC-3 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Twenty serum samples, sixteen EDTA plasma samples, and eighteen heparin plasma samples were evalutated for detectable levels of rat CINC-3 in this assay. All samples measured below the lowest standard, 31.3 pg/mL.

Cell Culture Supernates:
Rat lung conditioned media (1 lung, 1-2 mm pieces) was cultured for 3 days in 30 mL of RPMI supplemented with 10% fetal bovine serum and stimulated with 10 µg/mL ConA. An aliquot of the cell culture supernate was removed, assayed for rat CINC-3, and measured 16,060 pg/mL.

Rat heart conditioned media (1 heart, 1-2 mm pieces) was cultured for 4 days in 30 mL of RPMI supplemented with 10% fetal bovine serum and stimulated with 2.5 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed for rat CINC-3, and measured 12,004 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat CINC-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 rat CINC-3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:

CINC-1
CINC-2α
CINC-2β
CNTF
CNTF Rα
EphA5
EphB1
E-Selectin
Fractalkine
GDNF
GDNF Rα1
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1 R6
IL-1ra
IL-2
IL-4
IL-6
IL-10
IL-18
LIX
MAG
MIP-3α
β-NGF
Npn-1
Npn-2
PDGF-AA
PDGF-AB
PDGF-BB
TIMP-1
TNF-α
VEGF

Recombinant mouse:

MIP-2

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