

## **Rat CXCL1/CINC-1/KC ELISA Kit**

**Catalog Number: RK00196**

This ELISA kit used for quantitative determination of rat Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1) concentrations in cell culture supernates, serum and plasma. For research use only, and it's highly recommended to read throughly of this manual before using the product.

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## Introduction

Rat Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1), otherwise known as CXCL1, CINC, rat GRO $\alpha$ , and rat KC, is an 8 kDa member of the  $\alpha$ -, or CXC family of chemokines (1-4). It is synthesized as a 96 amino acid (aa) precursor that contains a 24 aa signal sequence plus a 72 aa mature segment (5, 6). In the mature segment, there are two intrachain disulfide bonds plus an ELR and CXC motif. There are no potential N-linked glycosylation sites. The molecule reportedly circulates as a nondisulfide-linked homodimer (1, 5, 7). In the mature region, rat CINC-1 shows 66% and 70% aa sequence identity to rat CINC-2 $\alpha$  and CINC-3, respectively (1, 5, 8, 9). It shows 92% aa sequence identity to mouse KC and 69% aa sequence identity to human GRO $\alpha$  (5, 10). Cells known to express CINC-1 are varied and include hepatocytes (11, 12), mast cells (13), macrophages (14-16), neurons (17), Kupffer cells (12), fibroblasts (18), type II greater alveolar cells (19), and cardiac muscle cells (20). The receptor for rat CINC-1 is CXCR2 (21, 22). Although both rat CXCR1-like and CXCR2 have been reported in the literature (22), the CXCR1-like molecule is unresponsive to CXC ligands. Thus, only CXCR2 is considered the functional CINC receptor (21). Functionally, CINC-1 is described as a major neutrophil chemoattractant and activator (1, 13, 23-25). CINC-1, induced by IL-1 $\beta$ , TNF- $\alpha$  and bacterial products, promotes both neutrophil rolling and adhesion, likely through the upregulation of surface integrins (15, 25-28). Thus, it directs neutrophils to sites of bacterially-induced inflammation (27). It is also reported to stimulate neutrophil activity by promoting cathepsin G release from azurophilic granules (23). It does not, however, seem to impact nitrite production (24). Thus it makes a partial contribution to bacterial killing. Relative to CINC-2 $\alpha$  and CINC-3,

**CINC-1 seems to be equal in chemotactic activity but less efficient in inducing calcium mobilization (29). It is also induced earlier in macrophages than CINC-2 and -3 and declines more quickly in expression (16). The significance of this is unclear.**

## Principle Of The Assay

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for CXCL1/CINC-1/KC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CXCL1/CINC-1/KC present is bound by the immobilized antibody. Following incubation unbound samples are removed during a wash step, and then a detection antibody specific for CXCL1/CINC-1/KC is added to the wells and binds to the combination of capture antibody- CXCL1/CINC-1/KC in sample. Following a wash to remove any unbound combination, and enzyme conjugate is added to the wells. Following incubation and wash steps, a substrate is added. A colored product TMB is formed in proportion to the amount of CXCL1/CINC-1/KC present in the sample. The reaction is terminated by addition of acid and absorbance is measured. A standard curve is prepared from seven CXCL1/CINC-1/KC standard dilutions and CXCL1/CINC-1/KC sample concentration determined.

## Materials Provided

Part	Size (96T)	Cat NO.	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Antibody Coated Plate	8×12	RM00812	Return unused wells to the foil pouch containing the desiccant pack and store at ≤ -20 °C. Reseal along entire edge of zip-seal.
Standard Lyophilized	3	RM00809	Aliquot and store at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Concentrated Biotin Conjugate Antibody (30×)	1 ×400ul	RM00810	May be stored for up to 6 month at 2-8 °C.*
Streptavidin-HRP Concentrated (30×)	1 ×400ul	RM00811	
Standard/Sample Diluent (R1)	1 ×20mL	RM00023	May be stored for up to 6 month at 2-8 °C.*
Biotin-Conjugate Antibody Diluent (R2)	1 ×16mL	RM00024	
Streptavidin-HRP Diluent(R3)	1 ×16mL	RM00025	
Wash Buffer(20×)	1 × 30mL	RM00026	
TMB Substrate	1 ×12 mL	RM00027	
Stop Solution	1 ×12 mL	RM00028	

<b>Plate Sealers</b>	<b>4 strips</b>
<b>Specification</b>	<b>1</b>

## Sample Collection And Storage

### 1. Cell Culture Supernates:

Centrifuge 1000x g for 10 min and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles. If cell culture supernate samples require larger dilutions, perform an intermediate dilution with culture media and the final dilution with the Standard/Sample Diluent(R1).

### 2. Serum:

Use a serum separator tube and allow samples to clot for 30 minutes before centrifugation for 10 minutes at 1000x g, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze/thaw cycles.

### 3. Plasma

Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000x g within 30 minutes of collection, and detect; or aliquot and store samples at -20°C to -70°C (Stored at 2-8°C if tested within 24 hours). Avoid freeze / thaw cycles.

### 4. Avoid hemolytic and hyperlipidemia sample for Serum and Plasma.

### 5. Dilution:

Dilute samples at the appropriate multiple (recommend to do pre-test to determine the dilution factor).

## Precautions

1. **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
2. Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
3. Variations in sample collection, processing, and storage may cause sample value differences.
4. Reagents may be harmful, if ingested, rinse it with an excess amount of tap water.
5. Stop Solution contains strong acid. Wear eye, hand, and face protection.
6. Apart from the standard of kits, other components should not be refrigerated.
7. Please perform simple centrifugation to collect the liquid before use.
8. Do not mix or substitute reagents with those from other lots or other sources.
9. Adequate mixing is very important for good result. Use a mini-vortexer at the lowest frequency.
10. Mix the sample and all components in the kits adequately, and use clean plastic container to prepare all of the diluent.
11. Both the sample and standard should be assayed in duplicate, and the sequence of the reagents should be added consistently.
12. Reuse of dissolved standard is not recommended.
13. The kit should not be used beyond the expiration date on the kit label.

14. The kit should be away from light when it is stored or incubated.
15. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.
16. To avoid cross contamination, please use disposable pipette tips.
17. Please prepare all the kit components according to the Specification. If the kits will be used several times, please seal the rest strips and preserve with desiccants. Do use up within 2 months.
18. The 48T kit is also suitable for the specification.

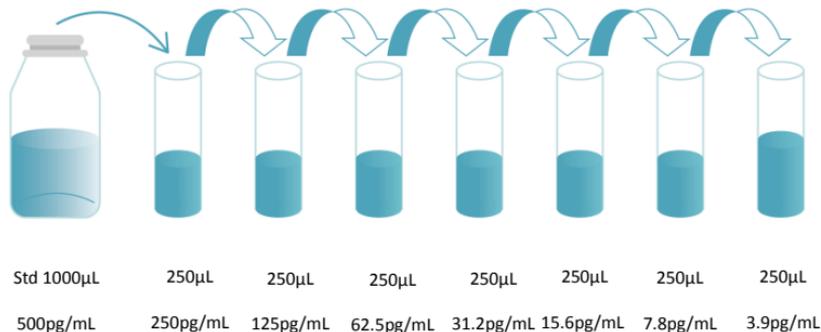
## Experiment Materials

1. Microplate reader(measuring absorbance at 450 nm, with the correction wavelength set at 570 nm or 630 nm).
2. Pipettes and pipette tips: 0.5-10, 2-20, 20-200, 200-1000  $\mu$ L.
3. Microplate washer, Squirt bottle.
4. Micro-oscillator.
5. Deionized or double distilled water, graduated cylinder.
6. Polypropylene Test tubes for dilution.
7. Incubator.

## Reagent Preparation

- 1. Bring all reagents to room temperature before use. If crystals have formed in the concentrate, Bring the reagent to room temperature and mix gently until the crystals have completely dissolved.**
- 2. Standard:** Add Standard/Sample Diluent(R1) 1.0mL into freeze-dried standard, sit for a minimum of 15 minutes with gentle agitation prior to making dilutions (500pg/mL), Prepare EP tubes containing Standard/Sample Diluent(R1), and produce a dilution series according to the picture shown below (recommended concentration for standard curve: 250, 125, 62.5, 31.2, 15.6, 7.8, 3.9, 0 pg/mL). Redissolved standard solution (500 pg/mL), aliquot and store at -20°C— -70°C.

Std 250 $\mu$ L      250 $\mu$ L



- 3. Concentrated Biotin Conjugate Antibody (30x)** : Dilute 1:30 with the Biotin-Conjugate Antibody Diluent (R2) before use, and the diluted solution should be used within 30 min.

### Dilution Method

Strip	Concentrated Biotin-Conjugate antibody (30x)	Biotin-Conjugate Antibody Diluent (R2)
2	66uL	1914uL
4	132uL	3828uL
6	198uL	5742uL
8	264uL	7656uL
10	330uL	9570uL
12	396uL	11484uL

4. **Streptavidin-HRP Concentrated (30x):** Dilute 1:30 with the Streptavidin-HRP Diluent(R3) before use, and the diluted solution should be used within 30 min.

### Dilution Method

Strip	Concentrated Streptavidin-HRP (30x)	Streptavidin-HRP Diluent(R3)
2	66uL	1914uL
4	132uL	3828uL
6	198uL	5742uL
8	264uL	7656uL
10	330uL	9570uL
12	396uL	11484uL

5. **Wash buffer:** Dilute 1:20 with double distilled or deionized water before use.

## Wash Method

Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with **Wash Buffer**(300ul) 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.

## Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add wash buffer 300  $\mu$ L/well, aspirate each well after holding 40 seconds, repeating the process two times for a total of three washes.
3. Add 100  $\mu$ L Standard/sample Diluent (R1) in blank well.
4. Add 100  $\mu$ L different concentration of standard and sample in other wells, cover with the adhesive strip provided. Incubate for 2 hours at 37°C.
5. Repeat the aspiration/wash as in step 2.
6. Prepare the Concentrated Biotin Conjugate Antibody (30X) Working Solution 15 minutes early before use.
7. Add Biotin-Conjugate Antibody Diluent(R2) in blank well and Biotin-Conjugate antibody Working Solution in other wells (100 $\mu$ L/well),

cover with new adhesive strip provided. Incubate for 1 hour at 37°C.

8. Prepare the Streptavidin-HRP Concentrated (30X) Working Solution 15minutes early before use.
9. Repeat the aspiration/wash as in step 2.
10. Add Streptavidin-HRP Diluent(R3) in blank well and add Streptavidin-HRP Working Solution in other wells (100  $\mu$ L/well), cover with new adhesive strip provided. Incubate for 30 minutes at 37°C.
11. Warm-up the Microplate reader.
12. Repeat the aspiration/wash as in step 2.
13. Add TMB Substrate (100 $\mu$ L/well). Incubate for 15-20 minutes at 37°C .Protect from light.
14. Add Stop Solution (50 $\mu$ L/well), determine the optical density of each well within 5 minutes, using a Microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 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.

## Assay Procedure Summary

**Prepare the standard and reagents**



**Wash plate 3 times**



**Add 100ul of standards and test samples to each well**

*Incubate for 2 hours at 37°C* ↓ *wash 3 times*

**Add 100ul Biotin-Conjugate antibody Working Solution**

*Incubate for 1 hour at 37°C* ↓ *wash 3 times*

**Add 100ul Streptavidin-HRP Working Solution**

*Incubate for 30 min at 37°C* ↓ *wash 3 times*

**Add 100ul Substrate Solution**

*Incubate for 15-20 min* ↓ *at 37°C under dark condition.*

**Add 50ul Stop Solution**

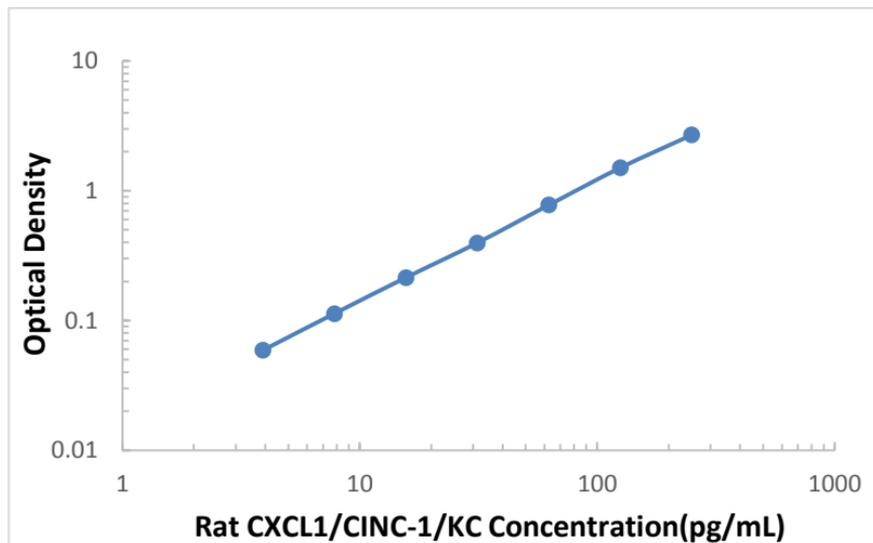
**Detect the optical density within 5 minutes under 450nm.**

**Correction Wavelength set at 570nm or 630nm**

## Calculation Of Results

1. Average the duplicate readings for each standard, control and sample, and subtract the average zero standard optical density (O.D.).
2. 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 CINC-1 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.
3. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## Typical Data



The standard curves are provided for demonstration only. A standard curve should be generated for each set of CXCL1/CINC-1/KC assayed.

## Sensitivity

The minimum detectable dose (MDD) of CXCL1/CINC-1/KC is typically less than 1.95 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.

## Specificity

This assay recognizes both recombinant and natural rat CXCL1/CINC-1/KC. The factors listed below were prepared at 50ng/ml and assayed for cross-reactivity. No significant cross-reactivity was observed with the following:

Does not react with other soluble molecules

## Precision

### Intra-plate Precision

Three samples of known concentration were tested 20 times on one plate to evaluate the Intra-plate precision.

<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>
<b>Repeat Times</b>	<b>20</b>	<b>20</b>	<b>20</b>
<b>Average Value (pg/mL)</b>	<b>60</b>	<b>490</b>	<b>950</b>
<b>Standard Deviation (SD)</b>	<b>2.4</b>	<b>17.1</b>	<b>36.1</b>
<b>Variable Coefficient CV (%)</b>	<b>4.1</b>	<b>3.5</b>	<b>3.8</b>

### Inter-plate Precision

Three samples of known concentration were tested 20 times separate assays to evaluate the Inter-plate precision. Assays were using two lots of components.

<b>Sample</b>	<b>1</b>	<b>2</b>	<b>3</b>
<b>Repeat Times</b>	<b>20</b>	<b>20</b>	<b>20</b>
<b>Average Value (pg/mL)</b>	<b>75</b>	<b>450</b>	<b>920</b>
<b>Standard Deviation (SD)</b>	<b>4.7</b>	<b>31.9</b>	<b>62.5</b>
<b>Variable Coefficient CV (%)</b>	<b>6.3</b>	<b>7.1</b>	<b>6.8</b>

## Recovery

Spike 3 different concentration of rat CXCL1/CINC-1/KC into healthy rat serum and plasma, calculate the recovery.

Sample Form	Average Recover (%)	Range (%)
Serum	98	86-115
Plasma	100	83-106

## Linearity

Spike high concentration of rat CXCL1/CINC-1/KC into 4 healthy rat serum, dilute in the range of standard curve kinetics and evaluate the linearity.

Dilution	Average Value (%)	Range (%)
1:2	98	85-113
1:4	97	86-115
1:8	97	90-120
1:16	98	82-108

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