

## Pana-test Rat CINC-1 (IL-8)

### 1. Introduction

CINC-1 (cytokine-induced neutrophil chemoattractant) was found in the culture media of NRK-52E cell, that was a cell line established from a normal rat kidney. There are several types of CINC, such as CINC-1, CINC-2 $\alpha$ , CINC-2 $\beta$ , and CINC-3. Recently, CINC-1 has been confirmed to be identical with IL-8. CINC-1, a peptide molecule (MW= 7854 kDa), is involved in inflammatory reactions by the promotion of the neutrophil migration and induction of the enzyme releasing from neutrophil.

### 2. Characteristics

- This kit includes an exclusive reagent for quantitative determination of CINC-1.
- No specific facility is necessary.

### 3. Components of the Kit

- ELISA plate (anti-rat CINC-1 antibody-coated microplate)..... 1 plate
- Standard rat CINC-1 (1,600 pg/mL) for 2 mL (lyophilized) ..... 1 vial
- Sample diluent, 40 mL ..... 1 vial
- Enzyme-labeled antibody (peroxidase-conjugated anti-rat CINC-1 antibody)  
for 12 mL (lyophilized)..... 1 vial
- Chromogen solution (containing 13.2 mg of 3,3',5,5'-tetramethylbenzidine in 0.5 mL of  
*N,N*-dimethylformamide) ..... 1 vial
- Substrate solution, 20 mL (containing 0.0083 w/v% hydrogen peroxide)..... 1 vial
- Washing buffer concentrate, 40 mL (10-fold concentrated PBS-Tween 20, for 400 mL use)..... 1 vial
- Stop solution, 15 mL (1 mol/L sulfuric acid)..... 1 vial

#### 4. Reagent Preparation

Component	Preparation	Reagent prepared	Storage condition and stability
① ELISA plate	Wait until the plate reaches to room temperature. Add 300 $\mu$ L of wash buffer to each well just before use, and leave for 10 minutes.	Anti-rat CINC-1 antibody-coated plate	Prepare a required number of strip only immediately before use.
② Standard rat CINC-1	Add accurately 2.0 mL of purified water <sup>1)</sup> to vial, and mix it thoroughly for complete dissolution. Be careful not to be bubbled.	Standard rat CINC-1 (1,600 pg/mL)	Stable in a refrigerator (2 to 10°C) for one week
③ Sample diluent	Use it as it is		Stable in a refrigerator (2 to 10°C) for one week
④ Enzyme-labeled antibody	Add accurately 12 mL of purified water to vial, and mix it thoroughly.	Enzyme-labeled antibody solution	Stable in refrigerator (2 to 10°C) for one week
⑤ Chromogen solution ⑥ Substrate solution	Add 100 $\mu$ L of the chromogen solution into 10 mL of the substrate solution.	Chromogenic substrate solution	Freshly prepare, just before use
⑦ Wash buffer concentrate	Add the entire volume of the concentrate (40 mL) into 360 mL of purified water, and mix it thoroughly.	Wash buffer <sup>2)</sup>	Stable at room temperature for one week
⑧ Stop solution	Use it as it is		Stable at room temperature

1) Distilled or deionized water

2) PBS containing 0.05 v/v% Tween 20

NOTE: \*: Distilled or deionized water

All reagents should be allowed to equilibrate to room temperature before use.

Disused strips should be closed up in the foil pouch and stored at 2 to 10°C under dark.

Immediately use the chromogenic substrate solution after mixing ⑤ with ⑥.

#### 5. Supplies Required

- Micropipettes and pipette tips (50  $\mu$ L, 100 to 1,000  $\mu$ L)
- Blowout pipettes (2 mL, 10 mL)
- Graduated cylinder (500 mL)
- Squirt bottle, manifold dispenser, or automated microplate washer
- Multi-channel pipette
- Microplate reader capable of measurement at or near 450 nm
- Distilled or deionized water

## 6. Assay Procedure

### 6.1 Preparation of Standard Rat CINC-1 Solutions

Reconstitute the standard rat CINC-1 with accurately 2.0 mL of deionized or distilled water, producing 1,600 pg/mL standard. Swirl or mix gently and leave for a while to ensure complete reconstitution. Make serial dilutions of the 1,600 pg/ mL standard with the sample diluent, to prepare the standard solutions at 800, 400, 200, 100, 50, 25 and 12.5 pg/mL. Distribute the sample diluent to the 0 pg/mL standard.

### 6.2 Sample Dilution

#### Blood sample

Commonly use serum or plasma (containing heparin or EDTA as an anti-coagulant) as a sample. Sample should be diluted with the sample diluent over 4-fold. Store the samples below -20°C. Culture medium samples, containing fetal call serum up to 10%, can be provided to the CINC-1 assay with no dilution.

### 6.3 Assay Protocol

Bring all reagents and samples to room temperature before use. It is recommended that all samples, including the standards, be assayed in duplicate.

- 1) Add 300  $\mu$ L of the wash buffer to each well of the ELISA plate. Incubate for 10 minutes at room temperature. (no adverse effect, even when left standing for up to 30 minutes.)
- 2) Aspirate each well to remove the solution.
- 3) Add 100  $\mu$ L of the standard rat CINC-1 solution or unknown samples to each well, and incubate for 2 hours at room temperature.
- 4) Aspirate each well and wash with wash buffer (300  $\mu$ L/well), repeating the washing procedure further twice. Complete removal of liquid in each wash is essential to good performance.
- 5) Add 100  $\mu$ L of the enzyme-labeled antibody solution to each well, and incubate for 1 hour at room temperature.
- 6) Wash the wells as in step 4.
- 7) Add 100  $\mu$ L of the chromogenic substrate solution to each well and incubate at room temperature for 30 minutes.
- 8) Add 50  $\mu$ L of the stop solution to each well.
- 9) Measure the absorbance at 450 nm ( $A_{450}$ ) with a microtiter plate reader.

## 7. Calculation of results

- 1) Average the duplicate reading for each standard and sample.
- 2) Plot the values of  $A_{450}$  (Y-axis) versus the concentrations of the standard solutions (X-axis), thus draw the standard curve.
- 3) Apply an  $A_{450}$  value of each sample in the standard curve, so as to read the rat CINC-1 concentration in the sample.
- 4) Multiply the CINC-1 concentration by the dilution factor to get a CINC-1 concentration in the serum sample.

## 8. Safety Warnings and Precautions

- Strictly observe the storage condition for each reagent.
- All reagents should be brought to room temperature before use.
- Use reagents after confirming complete dissolution and uniformity.
- Take care to not inflict damage on any well when aspirating the solution in each well.
- When measuring many samples in one assay batch, the time period of each reaction for all samples should be uniformed at a fixed time as designated.
- Prepare the standard curve freshly for every measurement.
- Prepare the substrate solution with a clean vessel.
- White powder may sometimes be found in the wells of ELISA plate. This is due to the dried blocking solution, but will have no effect on measurement.
- Take care to handle the stop solution, very harmful.

## 9. Performance Characteristics

### 9.1 Quantitative Range

12.5 – 800 pg/mL of rat CINC-1

### 9.2 Intra - assay Precision

#### Standards

Rat CINC-1 (pg/mL)	(Replicate)	A <sub>450</sub> (mean)	C.V. (%)
0	(N=6)	0.065	1.5
12.5	(N=6)	0.110	1.8
25	(N=6)	0.154	1.9
50	(N=6)	0.243	2.5
100	(N=6)	0.429	2.8
200	(N=6)	0.752	1.2
400	(N=6)	1.354	2.9
800	(N=6)	2.221	2.5

#### Samples

Sample	(Replicate)	A <sub>450</sub>		CINC-1 conc.	
		mean	CV (%)	mean	CV (%)
A	(N=6)	0.323	1.9	69	2.7
B	(N=6)	1.495	4.3	460	5.8

C.V. = coefficient of variation

Sample A and B: Rat plasma (containing heparin) spiked with standard rat CINC-1

### 9.3 Inter-assay Precision

#### Standards

Rat CINC-1 (pg/mL)	(Replicate)	A <sub>450</sub> (mean)	C.V. (%)
0	(N=6)	0.066	5.5
12.5	(N=6)	0.113	3.4
25	(N=6)	0.161	3.0
50	(N=6)	0.256	2.4
100	(N=6)	0.444	2.5
200	(N=6)	0.788	4.0
400	(N=6)	1.431	3.3
800	(N=6)	2.294	4.9

#### Samples

Sample	(Replicate)	A <sub>450</sub>		CINC-1 conc. (pg/mL)	
		mean	CV (%)	mean	CV (%)
A	(N=6)	0.311	3.0	65	4.7
B	(N=6)	1.452	3.2	416	5.6

C.V. = coefficient of variation

Sample A and B: Rat plasma (addition of heparin) spiked with standard rat CINC-1

### 9.4 Recovery

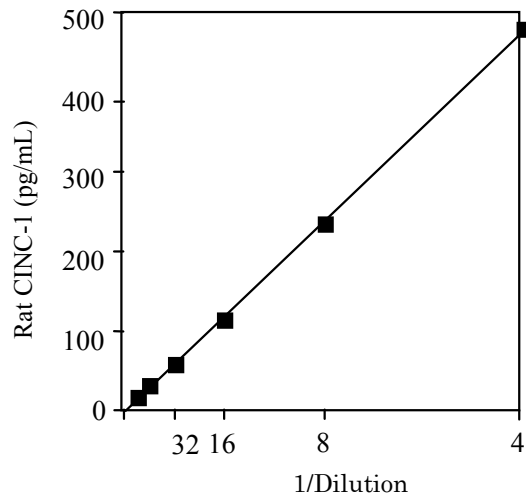
Samples were prepared by spiking standard rat CINC-1 into the diluted samples

Sample	Dilution	
	1/4	1/8
Heparin-plasma	90 – 113%	103 – 117%
EDTA-plasma	98 – 115%	99 – 142%
Sodium citrate-plasma	83 – 99%	75 – 97%
Serum	92 – 125%	94 – 103%

Sample	Dilution	
	1	1/2
DMEM medium containing 10% FCS	99 – 114%	99 – 100%

## 9.5 Linearity of Dilution

Samples were prepared by a serial dilution of a rat serum with the sample diluent from 4 to 128-fold.



## 10. Storage and Expiry

Store all reagents at 2-10°C under dark and use until a stated expiration date (one year after manufactured).

## 11. Package

96 tests per kit

<Distributed by>

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