



KAMIYA BIOMEDICAL COMPANY

Rat CINC-1 ELISA

For the quantitative determination of CINC-1 in rat serum and plasma

Cat. No. KT-451

For research use only, not for use in diagnostic procedures.

PRODUCT INFORMATION

Rat CINC-1 ELISA **Cat. No. KT-451**

PRODUCT

The **K-ASSAY®** Rat CINC-1 ELISA is for the quantitative determination of CINC-1 in rat serum and plasma.

PRINCIPLE

CINC-1 (cytokine-induced neutrophil chemoattractant) is refined from the supernatant of NRK-52E cultivation, which is a normal rat kidney cell type. There are many kinds of CINC, such as CINC-1, CINC-2 α , CINC-2 β , and CINC-3. Later, CINC-1 was confirmed to be the same as IL-8. CINC-1 is a peptide with the molecular weight of 7,845, and it is a factor that promotes the migration of neutrophils. Since CINC-1 has proved that it induces the release of cellular enzymes, it is also noted to be involved in inflammation. Since this kit includes an exclusive reagent for quantitative determination in rats, specific and precise data can be obtained. The ELISA method does not require any special facility.

COMPONENTS

- Microtiter Plate: Anti-rat CINC-1 antibody-coated solid phase plate, 96-wells
- Rat CINC-1 Calibrator: 1,600 pg/mL for 2 mL (lyophilized)
- Sample Diluent: 40 mL
- Enzyme-labeled antibody: Peroxidase-conjugated anti-rat CINC-1 antibody, 12 mL (lyophilized)
- Chromogen Solution: 13.2 mg 3,3',5,5'-tetramethylbenzidine in 0.5 mL N,N-dimethylformamide
- Substrate Solution: 0.0083 w/v% of hydrogen peroxide, 20 mL
- Concentrated Washing Solution: 10-fold concentrated PBS, Tween 20, 40 mL (for 400 mL use)
- Stop Solution: 1M Sulfuric Acid, 15 mL

Materials or Equipment required but not provided

- Micropipette and tips (50 μ L, 100-1,000 μ L)
- Mass pipette (2 mL, 10 mL)
- Mass cylinder (500 mL)
- Cleaning instrument for 96-well microtiter plate (In case of manual operation: continuous distributor aspirator, etc.)
- Microtiter plate reader (450 nm)
- Multi-channel pipette

PREPARATION OF REAGENTS

A. Test Sample

1. Use the supernatant of serum or plasma (with the addition of heparin or EDTA). Dilute test samples with diluent to 4 or more times. In case of fetal calf serum (FCS) concentration of culture supernatant is approximately 10%, it can measure with an undiluted solution. Keep samples below -20 °C.

B. Rat CINC-1 Calibrator

1. Accurately add 2.0 mL of purified water to the vial containing the rat CINC-1 calibrator, providing the concentration of 1,600 pg/mL.
2. Dilute the original solution in a series so as to prepare varying dilutions of 800, 400, 200, 100, 50, 25, and 12.5 pg/mL. For 0 pg/mL, use the sample diluent in its intact form. In case CINC-1 is at a low concentration, it may stick to the glass. Dilute it in polypropylene tubes.

PROCEDURE

1. It is recommended to conduct all measurements in duplicity or in higher multiplicity.
2. Add 300 µL of wash buffer into each well of the ELISA plate. Incubate for 10 mins at room temperature. (There is no adverse affect even if left standing for up to 30 mins.)
3. Aspirate the solution
4. Add 100 µL of rat CINC-1 calibrator or the unknown sample into each well. Incubate for 2 hours at room temperature.
5. Aspirate the solution and wash well 3 times with wash buffer 300 µL/well/wash. Aspirate and top firmly after each wash to remove residual buffer.
6. Add 100 µL of enzyme-labeled antibody into each well. Incubate at room temperature for one 1 hour.
7. Repeat step 5.
8. Add 100 µL of chromogenic substrate solution into each well and incubate at room temperature for 30 mins.
9. Add 50 µL of stop solution into each well.
10. Measure the absorbance at 450 nm with a microtiter plate reader.

CALCULATION OF RESULTS

1. Average the duplicate readings for each calibrator and test sample.
2. Plot the concentration of the calibrator solution on the X-axis and the value of the absorbance on the Y-axis to prepare the calibration curve.
3. Apply the values of the absorbance of the test sample into the calibration curve, so as to read the rat CINC-1 concentration in the test sample and multiply this concentration by the dilution factor.

TECHNICAL HINTS

- Strictly observe the term and method of storage for each test reagent.
- All reagents should be brought to room temperature before use.
- Use each test reagent after confirming that each of them is completely dissolved.
- Take care to not inflict damage to any well when aspirating the solution in each well.
- For measurement of many test samples, take care that the reaction time of each test sample is at a fixed time as designated.
- Prepare the calibration curve freshly for every measurement.
- Prepare the chromogenic substrate solution with a clean instrument before use.
- White powder may sometimes be found on the wells. This is due to the dried blocking solution, but will not have an effect on measurement.
- As the stop solution is 1M sulfuric acid, be cautious when using it.

PERFORMANCE CHARACTERISTICS

1. Intra- assay precision

Standard		
Rat CNIC-1 (pg/mL)	Mean value of abs.	(%) 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
Sample		
Plasma	Mean value of abs.	(%) C.V.
A (N=6)	0.323	1.9
B (N=6)	1.495	4.3
Plasma	Mean value of conc.(pg/mL)	(%) C.V.
A (N=6)	69	2.7
B (N=6)	460	5.8

(%) C.V.= coefficient of variation

2. Inter – assay precision

Standard		
Rat CNIC-1 (pg/mL)	Mean value of abs.	(%) 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
Sample		
Plasma	Mean value of abs.	(%) C.V.
A (N=6)	0.311	3.0
B (N=6)	1.452	3.2
Plasma	Mean value of conc.(pg/mL)	(%) C.V.
A (N=6)	65	4.7
B (N=6)	416	5.6

(%) C.V.= coefficient of variation

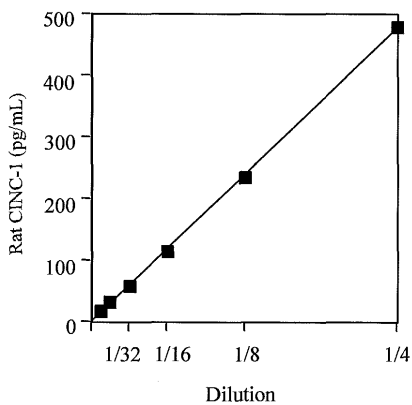
3. Test of recovery after addition

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%

The results of measurement with samples of SD rats to which the rat CINC-1 calibrator was added are shown above.

4. Dilution Test



Linearity with dilution can be obtained within the range of 4 to 128 fold dilution of urine samples of SD rats (male, 7 weeks of age).

5. Specificity

Testing shows undetectable cross-reactivity with rat CINC-2 α , CINC-2 β , and MIP-2 (below 0.002%).

STORAGE

Store at 4°C in a dark and cool place. The kit is stable as supplied until the expiration date.

FOR RESEARCH USE ONLY**KAMIYA BIOMEDICAL COMPANY**

12779 Gateway Drive, Seattle, WA 98168

Tel: (206) 575-8068 Fax: (206) 575-8094

Email: LifeScience@k-assay.com

www.k-assay.com