Rat CINC-1 ELISA

Cat. No.: RSCPRI121R

1. INTRODUCTION

CINC-1 (cytokine-induced neutrophil chemoattractant) is refined from supernatant of NRK-52E cultivation, which normal rat kidney cell type. There are some kinds in CINC, each as CINC-1, CINC-2α, CINC-2β, and CINC-3. Latterly CINC-1 was confirmed as same as IL-8. CINC-1 is peptide of molecular weight of 7845, and it is a factor to promote migration of neutrophil. Since CINC-1 has proved that it induces release of cellular enzyme, it is also noted that it involves to inflammation.

2. CHARACTERISTICS

1. Since this includes an exclusive reagent for quantitative determination in rats, specific and precise data can be obtained.
2. Because of employing the EIA method. no special facility is.

3. COMPOSITION OF THE REAGENT KIT

1. ELISA plate (Anti-rat CINC-1 antibody-coated solid phase plate) 1 plate
2. Standard rat CINC-1 (1600 pg/mL) for 2 mL (lyophilized) 1 vial
3. Sample diluent 40 mL 1 vial
4. Enzyme-labeled antibody (Peroxidase-conjugated rat CINC–1 antibody) for 12 mL use (lyophilized) 1 vial
5. Chromogen solution (Containing 13.2 mg of 3,3',5,5'-Tetramethylbenzidine in 0.5 mL N,N-dimethylformamide) 1 vial
6. Substrate solution (Containing 0.0083 w/v% of hydrogen peroxide) 20 mL 1 vial
7. Wash buffer concentrate 40 mL (10-fold concentrated PBS-Tween 20, for 400 mL use) 1 vial
8. Stop solution 15 mL (1 mol/L sulfuric acid) 1 vial

4. REAGENT PREPARATION

Reagent	Method for preparation	Reagent prepared	Method and terms for valid storage
1. ELISA plate	Wait until the plate return to room temperature. Add 300 µL of wash buffer into each wells just before use, and leave them for 10 minutes.	Anti-rat CINC-1antibody-coated plate.	Prepare a necessary strips number, before use.
2 Standard rat CINC-1	Accurately add 2.0 mL of purified water*, and mix in thoroughly for complete dissolution. Be careful not to bubble.	Standard rat CINC-1 (1600 pg/mL)	Stable in refrigerator (2-10°C) for one week.
3. Sample diluent buffer	Add the whole volume of 40 mL into 160 mL of purified water, and mix it thoroughly.	Sample diluent	Stable in refrigerator (2-10°C).
4. Enzyme-labeled antibody	Accurately add 12 mL of purified water to vial, and mix thoroughly.	Enzyme-labeled antibody solution	Stable in refrigerator (2-10°C) for one week.
5. Chromogen solution 6. Substrate reagent	Collect 10.0 mL of the substrate solution. Add 100 µL of chromogenic solution to it.	Chromogenic substrate solution	Freshly prepare it, just before use.
7. Wash buffer concentrate	Add the whole volume of 40 mL into 360 mL of purified water, and mix thoroughly it.	Wash buffer (PBS-0.05 v/v% Tween 20)	Stable at room temperature for one week.
8. Stop solution	Use it as it is	----------	Stable at room temperature.

NOTE: *: Distilled or deionized water

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

The unnecessary strips should be closed up in the foil pouch and stored at 2-10°C protected from light.

Do not store chromogenic substrate solution after mixing (5) with (6)

5. NECESSARY INSTRUMENT AND APPARATURES

1. Micro pipette and tips (50 µL, 100 – 1000 µL)
2. Mass pipette (1 mL,10 mL)
3. Mass cylinder (500 mL)
4. Cleaning instrument for 96 wells microtiter plate
   (In the case of manual operation: Continuous distributor aspirator, etc.)
5. Multi-channel pipette
6. Microtiter plate reader (With measuring wave length of 450 nm)

6. OPERATION METHOD FOR MEASUREMENT

6.1 Preparation of standard rat CINC-1 solution

Accurately add 2.0 mL of distilled or deionized water into the vial containingt the standard rat CINC-1, providing the concentration of 1600 pg/mL. Dilute the original solution in a series so as to prepare varying dilutions of 800, 400, 200, 100, 50, 25, 12.5 pg/mL. For 0 ng/mL, use the sample diluent buffer in its intact form. In case CINC-1 is low concentration, it may stick to glass. Dilute it in polypropylene tube.

6.2 Preparation of test samples

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

6.3 Assay protocol

It is recommended to conduct all the measurements in duplicity or in a higher multiplicity.

1. Add 300 µL of the wash buffer to each well of the ELISA plate. Incubate for 10 minutes at room temperature. (There is no adverse effect, even when it is left standing for up to 30 minutes.)
2. Aspirate the solution.
3. Add 100 µL of the standard rat CINC-1 or the unknown sample to each well, and incubate for 2 hours at room temperature.
4. 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.

5. Add 100 µL of the enzyme-labeled antibody to each well, and incubate for one hour at room temperature.
6. Wash step as in step 4.
7. Add 100 µL of the chromogenic substrate solution to each well and incubate at room temperature for 15 minutes.
8. Add 50 µL of the stop solution to each well.
9. Measure the absorbance at 450 nm with a microfilter plate reader

7. CALCULATION OF RESULTS

1. Average the duplicate reading for each standard, sample.
2. Plot the value of absorbance (Y-axis) against the concentration of the standard solution (X-axis), thus prepare the standard curve.
3. Apply the values of the absorbance of the sample into the standard curve, so as to read the rat CINC-1 concentration in the sample, and multiply this concentration by the dilution multiple.

8. SAFETY WARNINGS AND PRECAUTIONS

1. Strictly observe the term and the method for storage for each reagent.
2. All reagents should be brought to room temperature before use.
3. Use reagent after confirming that each of them is completely dissolved.
4. Take care to not inflict damage to any well when aspirating the solution in each well.
5. For measurement of many samples, take care that the reaction time of each sample is at a fixed time as designated.
6. Prepare the standard curve freshly for every measurement.
7. Prepare the chromogenic substrate solution with a clean instrument before use. (The substrate reagent may be developed due to contamination of the instrument.)
8. White powder may sometimes be found on the wells. This is due to the dried block solution, but will not have an effect on measurement
9. As the stop solution is 1 mol/L sulfuric acid, take care to handle it.

9. PERFORMANCE OF THE SYSTEM

9.1 Range of measurement

Within the range of 12.5–800 ng/mL, rat CINC-1 can be measured with this system.

9.2 Intra - assay precision

standard			
Rat CNIC-1 (pg/mL)		Mean valueof absorbance	(%) 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 valueof absorbance	(%) C.V.
A	(N=6)	0.323	1.9
B	(N=6)	1.495	4.3
Plasma		Mean valueof absorbance	(%) C.V.
A	(N=6)	69	2.7
B	(N=6)	460	5.8

(%) C.V.= coefficient of vatiation

9.3 Inter - assay precision

standard			
Rat CNIC-1 (pg/mL)		Mean valueof absorbance	(%) 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 valueof absorbance	(%) C.V.
A	(N=6)	0.311	3.0
B	(N=6)	1.452	3.2
Plasma		Mean valueof absorbance	(%) C.V.
A	(N=6)	65	4.7
B	(N=6)	416	5.6

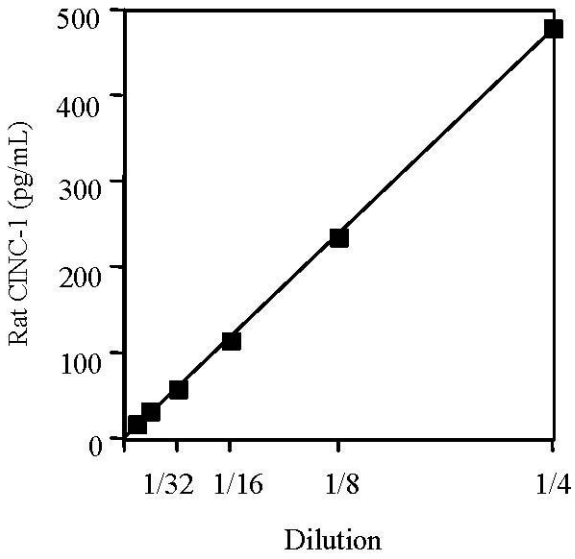
(%) C.V.= coefficient of vatiation

9.4 Test of recovery after addition

The results of measurement with samples of SD rats to which the standard rat CINC-1 was added were as shown below.

Dilution Sample	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 %

Dilution Sample	1/4	1/8
DMEM medium containing 10 % FCS	99-114 %	99-100 %



9.5 Dilution test

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

9.6 Specificity

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

10. METHOD FOR STORAGE AND TERMS OF VALIDITY

Stability is assured until the demonstrated expiration date (one year after manufacture), following storage in the dark and cool place (2 - 10°C).

11. PACKAGE

96 units for test.

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium
http://www.gentaur-worldwide.com