Reagent kit for measurement of Rat CINC-1 (IL-8)
For research purpose only

Prepared in July 2008
PRI121

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 CINCs, such each as CINC-1, CINC-2α, CINC-2β, 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 µL 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 [1)] 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 µL 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 [2)]	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 µL, 100 to 1,000 µL)
- Blowout pipettes (2 mL, 10 mL)
- Graduated cylinder (500 mL)
- Squirt bottle, manifold dispenser, or autmated 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 µL 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 µL 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 µL/well), repeating the washing procedure further twice. Complete removal of liquid in each wash is essential to good performance.
5) Add 100 µL 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 µL of the chromogenic substrate solution to each well and incubate at room temperature for 30 minutes.
8) Add 50 µL 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_{450} (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_{450}		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_{450} (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_{450}		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

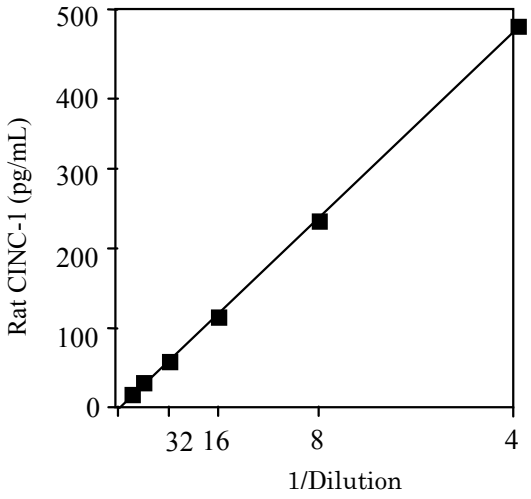
Samples were prepared by spiking standard rat CINC-1 into the diluted samples

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

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