

ab155462 – CINC-1 (CXCL1) Rat ELISA Kit

Instructions for Use

For the quantitative measurement of rat CINC-1 (CXCL1) in cell culture supernatants and plasma.

- △ **Notes:** Rat CINC-1 concentration is pretty low in normal plasma, it may not be detected in this assay. We have not been able to detect the endogenous Rat CINC-1 in normal serum with ab155462, only in serum spiked with Rat CINC-1.

This product is for research use only and is not intended for diagnostic use.

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1. BACKGROUND

Abcam's CINC-1 (CXCL-1) Rat ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay designed for the quantitative measurement of rat CINC-1 in plasma and cell culture supernatants. (Collect plasma using EDTA and heparin as an anticoagulant). Citrate is not recommended as anticoagulants for use in this assay. Rat CINC-1 concentration is pretty low in normal plasma, it may not be detected in this assay. We have not been able to detect the endogenous Rat CINC-1 in normal serum with ab155462, only in serum spiked with Rat CINC-1.

This assay employs an antibody specific for rat CINC-1 coated on a 96-well plate. Standards and samples are pipetted into the wells and CINC-1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-rat CINC-1 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of CINC-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

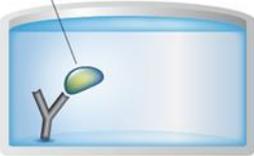
2. ASSAY SUMMARY

Primary capture antibody



Prepare all reagents, samples and standards as instructed.

Sample



Add standard or sample to each well used. Incubate at room temperature

Primary detector antibody



Add prepared biotin antibody to each well. Incubate at room temperature.

Streptavidin Label



Add prepared Streptavidin solution. Incubate at room temperature.

Substrate **Colored product**



Add TMB One-Step Development Solution to each well. Incubate at room temperature. Add Stop Solution to each well. Read immediately.

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C immediately upon receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 9 & 10.

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)
CINC-1 Microplate (12 x 8 wells)	96 wells	-20°C
20X Wash Buffer concentrate	25 mL	-20°C
Recombinant Rat CINC-1 Standard	2 vials	-20°C
5X Assay Diluent D	15 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Biotinylated anti-rat CINC-1	2 vials	-20°C
200X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 μ L to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

7. LIMITATIONS

- Do not mix or substitute reagents or materials from other kit lots or vendors.

8. TECHNICAL HINTS

- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.

- Complete removal of all solutions and buffers during wash steps. When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.
- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.
- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**

9. REAGENT PREPARATION

Equilibrate all reagents and samples to room temperature (18-25°C) prior to use.

9.1 1X Assay Diluent B

Dilute 5X Assay Diluent B 5-fold with deionized or distilled water before use.

9.2 1X Assay Diluent D

Dilute 5X Assay Diluent D 5-fold with deionized or distilled water before use.

9.3 1X Wash Buffer Solution

If the 20X Wash Buffer Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.4 1X Biotinylated CINC-1 Detection Antibody

Briefly spin the Biotinylated anti-rat CINC-1 vial before use. Add 100 μ L of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in Assay Procedure.

9.5 1X HRP-Streptavidin Solution

Briefly spin the 200X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 200X HRP-Streptavidin concentrate should be diluted 200-fold with 1X Assay Diluent B.

For example: Briefly spin the 200X HRP vial and pipette up and down to mix gently. Add 50 μ L of 200X HRP Streptavidin concentrate into a tube with 10 mL 1X Assay Diluent B to prepare a 200-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. STANDARD PREPARATIONS

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
 - Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.
- 10.1 Briefly spin the vial of CINC-1 Standard. Prepare a 50 ng/mL **Stock Standard** by adding 500 μL 1X Assay Diluent D or 1X Assay Diluent B into the vial.
 - 10.2 Ensure the powder is thoroughly dissolved by gentle mixing.
 - 10.3 Label tubes #1-8.
 - 10.4 **For Assay Diluent D:** Prepare **Standard #1** by adding 150 μL 50 ng/mL **Stock Standard** to 350 μL 1X Assay Diluent D into tube #1 (see Assay Diluent D table below).
 - 10.5 **For Assay Diluent B:** Prepare **Standard #1** by adding 30 μL 50 ng/mL **Stock Standard** to 470 μL 1X Assay Diluent B into tube #1 (see Assay Diluent B table below). As the 15,000 pg/mL standard in Assay Diluent B is saturated, we recommend starting from 3,000 pg/mL when using Assay Diluent B.
 - 10.6 Pipette 250 μL 1X Assay Diluent D or 1X Assay Diluent B into each tube.
 - 10.7 Prepare **Standard #2** by transferring 250 μL from tube #1 to tube #2, mix thoroughly.
 - 10.8 Prepare **Standard #3** by transferring 250 μL from tube #2 to tube #3, mix thoroughly.
 - 10.9 Using the appropriate table below as a guide, prepare further serial dilutions.
 - 10.10 Either 1X Assay Diluent B or 1 X Assay Diluent D serves as the zero standard, 0 ng/mL (Tube #8).

ASSAY PREPARATION

Standard Dilution Preparation Table Assay Diluent D

Standard #	Volume to Dilute (μL)	Diluent (μL)	Total Volume (μL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	150	350	500	50,000	15,000
2	250	250	500	15,000	7,500
3	250	250	500	7,500	3,750
4	250	250	500	3,750	1,875
5	250	250	500	1,875	937.5
6	250	250	500	937.5	468.8
7	250	250	500	468.8	234.4
8	0	250	250	0	0

Standard Dilution Preparation Table Assay Diluent B

Standard #	Volume to Dilute (μL)	Diluent (μL)	Total Volume (μL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	30	470	500	50,000	3,000
2	250	250	500	3,000	1,500
3	250	250	500	1,500	750
4	250	250	500	750	375
5	250	250	500	375	187.5
6	250	250	500	187.5	93.75
7	250	250	500	93.75	46.875
8	0	250	250	0	0



11. SAMPLE PREPARATION

General Sample Information:

- If your samples need to be diluted, Assay Diluent D should be used for dilution of plasma samples. 1X Assay Diluent B should be used for dilution of cell culture supernatants.
- Suggested dilution for normal plasma: 2 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

12. PLATE PREPARATION

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.
- Unused well strips should be returned to the plate packet and stored at 4°C.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).
- Well effects have not been observed with this assay.

13. ASSAY PROCEDURE

- **Equilibrate all materials and prepared reagents to room temperature (18 - 25°C) prior to use.**
- **It is recommended to assay all standards, controls and samples in duplicate.**

13.1. Add 100 μL of each standard (see Standard Preparations, section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

13.2. Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 μL) using a multi-channel Pipette 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.

13.3. Add 100 μL of 1X prepared biotinylated antibody (see Reagent Preparation, section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4. Discard the solution. Repeat the wash as in step 13.2.

13.5. Add 100 μL of prepared Streptavidin solution (see Reagent Preparation, section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6. Discard the solution. Repeat the wash as in step 13.2.

13.7. Add 100 μL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

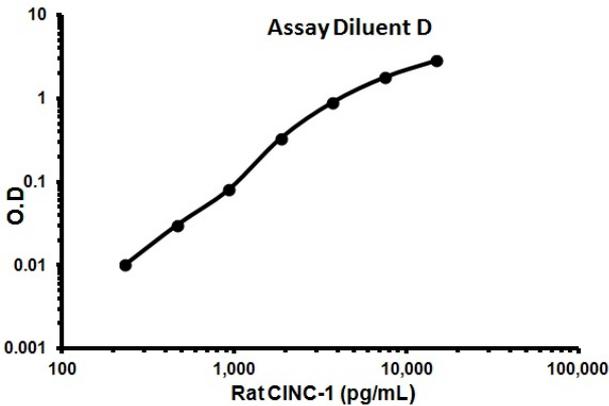
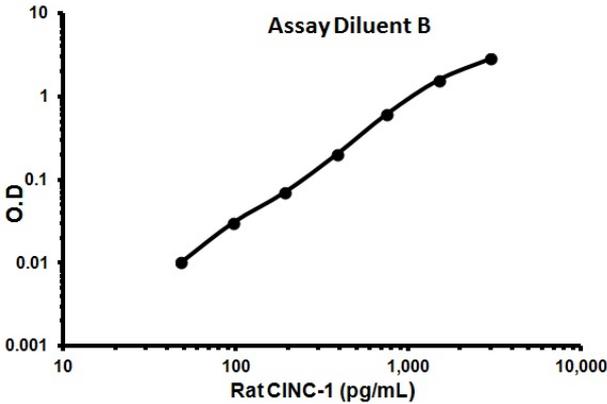
13.8. Add 50 μL of Stop Solution to each well. Read at 450 nm immediately.

14. CALCULATIONS

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D.	Conc. (pg/mL)	O.D.
	Assay Diluent B		Assay Diluent D
48.13	0.01	234.38	0.01
96.25	0.03	468.75	0.03
192.50	0.07	937.50	0.08
385.00	0.20	1,875.00	0.33
750.00	0.61	3,750.00	0.89
1,500.00	1.57	7,500.00	1.77
3,000.00	2.82	15,000.00	2.82

16. TYPICAL SAMPLE VALUES

SENSITIVITY –

The minimum detectable dose of CINC-1 is typically less than 300 pg/mL in Assay Diluent D and 50 pg/mL in Assay Diluent B.

RECOVERY –

Recovery was determined by spiking various levels of rat CINC-1 into rat plasma (EDTA and heparin) and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Plasma	107.9	97-125
Cell culture supernatant	112.5	99-122

LINEARITY OF DILUTION -

Plasma Dilution (EDTA and heparin)	Average % Expected Value	Range (%)
1:2	99.97	90-109
1:4	89.14	70-120

Cell Culture Supernatant Dilution	Average % Expected Value	Range (%)
1:2	82.27	74-90
1:4	75.54	67-82

PRECISION –

	Intra- Assay	Inter- Assay
CV	<10%	<12%

17. ASSAY SPECIFICITY

This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., rat CINC-2, CINC-3, CNTF, IL-1a, IL-1b, IL-6, IL-10, GM-CSF, IFN-g, Leptin, Lix, MCP-1, MIP-3a, b-NGF, TIMP-1, TNF-a, VEGF).

18. TROUBLESHOOTING

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

19. NOTES

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