

Code No. 27162

Rat GRO/CINC-1 Assay Kit - IBL

INTRODUCTION

Cytokine-induced neutrophil chemoattractant-1 (CINC-1) was originally purified from media conditioned by IL-1 β stimulated rat kidney epithelioid cells (NRK-52E). Watanabe's group at Toyama Medical and Pharmaceutical University identified amino acid sequence that encodes for rat CINC-1 in 1989. CINC-1 is a member of the alpha (CXC) subfamily of chemokines. Three additional rat CXC chemokines (CINC-2 α , CINC-2 β , CINC-3/MIP-2) have been identified. The protein sequence of CINC-1 is 63 - 67% identical to that of CINC-2 α , CINC-2 β , CINC-3/MIP-2. In addition, GRO α , GRO β and GRO γ is sharing 68%, 71% and 69%, identity with CINC-1. This has been suggested that CINCs are the rat counterpart of human GROs.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as coloring agent (Chromogen). The strength of coloring is in proportion to the quantities of Rat GRO/ CINC-1.

MEASUREMENT RANGE

4.69 ~ 300 pg/mL

INTENDED USE

- The IBL's Rat GRO/CINC-1 Assay Kit is a complete kit for the quantitative determination of GRO/CINC-1 in serum, EDTA-plasma and supernatant of cell culture media.
- Both recombinant and native forms of GRO/CINC-1 can be detected with the kit.

KIT COMPONENT

- | | | | |
|---|--------------------------------|--|------------|
| 1 | Precoated plate | : Anti- Rat GRO/CINC-1 Rabbit IgG Affinity Purify | 96Well x 1 |
| 2 | Labeled antibody Conc. | : (30X) HRP conjugated Anti-Rat GRO/CINC-1 Rabbit IgG Fab' Affinity Purify | 0.4mL x 1 |
| 3 | Standard | : Recombinant Rat GRO/CINC-1 | 0.5mL x 1 |
| 4 | EIA buffer | : 1% BSA, 0.05% Tween 20 in PBS | 30mL x 1 |
| 5 | Solution for Labeled antibody: | 1% BSA, 0.05% Tween 20 in PBS | 12mL x 1 |
| 6 | Chromogen | : TMB solution | 15mL x 1 |
| 7 | Stop solution | : 1N H ₂ SO ₄ | 12mL x 1 |
| 8 | Wash buffer Conc. | : (40X) 0.05% Tween20 in phosphate buffer | 50mL x 1 |

OPERATION MANUAL

1. Materials needed but not supplied

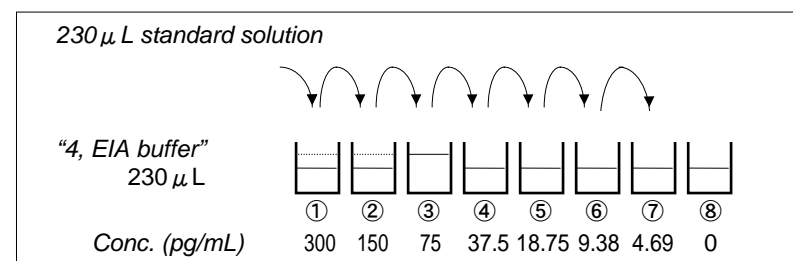
- Plate reader (450nm)
- Graduated cylinder and beaker
- Incubator (37°C \pm 1°C)
- Paper towel
- Washing bottle for precoated plate
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Distilled water
- Graph paper (log/log)
- Tube for dilution of Standard

2. Preparation

- 1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (X40) buffer. The temperature of "8, Wash buffer Conc." shall be adjusted to room temperature and then, mix it gently and completely before use. Dilute 50mL of "8, Wash buffer Conc." with 1,950mL of distilled water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
- 2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (X30). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one slit (8 well), the required quantity of Labeled antibody is 800 μ L. (Dilute 30 μ L of "2, Labeled antibody Conc." with 870 μ L of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 μ L in each well.)
This operation should be done just before the application of Labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard
Put just 0.5mL of distilled water into the vial of "3, Standard" and mix it gently and completely. This solution is 600 pg/mL Rat GRO/CINC-1 standard.
- 4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 μ L each of "4, EIA buffer" into the tube. Specify the following concentration of each tube.

Tube-1	300 pg/mL
Tube-2	150 pg/mL
Tube-3	75 pg/mL
Tube-4	37.5 pg/mL
Tube-5	18.75 pg/mL
Tube-6	9.38 pg/mL
Tube-7	4.69 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230 μ L of Standard solution into tube-1 and mix it gently. Then, put 230 μ L of tube-1mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 300 pg/mL and 4.69 pg/mL. Tube-8 is the test sample blank as 0 pg/mL. See following picture.



5) Dilution of test sample

Test sample should be diluted with "4, EIA buffer" as the need arises.

If the concentration of Rat GRO/CINC-1 in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Confirm no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

Reagents	Test Sample	Standard	Test Sample Blank	Reagent Blank
	Test sample 100 μ L	Diluted standard (Tube 1~7) 100 μ L	EIA buffer (Tube-8) 100 μ L	EIA buffer 100 μ L
Incubation for 1 hour at 37°C with plate lid				
Washing 7 times				
Labeled Antibody	100 μ L	100 μ L	100 μ L	-
Incubation for 30minutes at 37°C with plate lid				
Washing 9 times				
Chromogen	100 μ L	100 μ L	100 μ L	100 μ L
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 μ L	100 μ L	100 μ L	100 μ L
Read the plate at 450nm against a Reagent Blank within 30 minutes after application of Stop solution.				

- 1) Determine wells for reagent blank. Put 100 μ L each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 μ L each of test sample blank (tube-8), test sample and dilutions of standard (tube-1~7) into the appropriate wells.
- 3) Incubate the precoated plate for 1 hour at 37°C after covering it with plate lid.
- 4) Wash each well of the precoated plate vigorously with wash buffer using washing bottle. Then, fill each well with wash buffer and place the precoated plate for 15~30 seconds. Remove wash buffer completely from the precoated plate by snapping.
This procedure must be repeated more than 7 times.
Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
- 5) Pipette 100 μ L of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- 6) Incubate the precoated plate for 30 minutes at 37°C after covering it with plate lid.
- 7) Wash the precoated plate 9 times in the same manner above 4).
- 8) "6, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100 μ L from the test tube into the wells. Please avoid to return the rest of test tube into "6, Chromogen" bottle due to avoid to cause of contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "6, Chromogen".
- 10) Pipette 100 μ L of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "7, Stop solution".
- 11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450nm. The measurement shall be done within 30minutes after the addition of "7, Stop solution".

SPECIAL ATTENTION

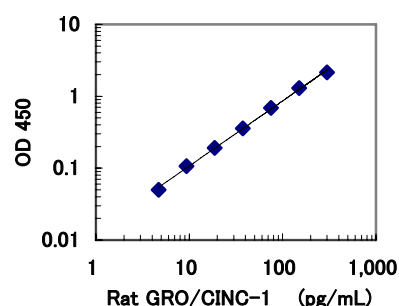
1. Test samples should be measured soon after the collection. In case of the storage of test samples, they should be stored under frozen conditions and do not repeat freeze/thaw cycles. Thaw the test samples at low temperature and mix them completely before measurement.
2. Test samples should be diluted with "4, EIA buffer", if the need arises.
3. The measurement of test samples and standard in duplicate is recommended.
4. Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
5. Use only wash buffer contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
6. Remove the wash buffer completely by tapping the precoated plate on paper towel.
Do not wipe wells with paper towel.
7. "6, Chromogen" should be stored in the dark due to its sensitivity against light. "6, Chromogen" should be avoided contact with metals.
8. Measurement should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

Conc. (pg/mL)	Absorbance (450nm)
300	2.173
150	1.333
75	0.720
37.5	0.385
18.75	0.219
9.38	0.135
4.69	0.078
0 (Test Sample Blank)	0.028



* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Titer Assay (Samples with standard added are used.)

Specimen	Titer (X)	Measurement Value (pg/mL)	Theoretical Value (pg/mL)	%
10% FCS added RPMI-1640	2	80.90	75.23	107.5
	4	39.84	37.60	106.0
	8	19.63	18.75	104.7
Rat Serum	2	189.81	204.97	92.6
	4	113.13	109.80	103.0
	8	57.20	56.37	101.5
Rat Plasma (EDTA) (Wistar)	4	65.79	72.33	91.0
	8	36.58	35.57	102.8
	16	18.34	17.89	102.5

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10% FCS added RPMI-1640 (x2)	75.09	72.73	96.9
	37.59	33.92	90.2
	18.84	16.91	89.8
Rat Serum (x4)	137.53	125.81	91.5
	100.03	92.07	92.0
	81.28	74.27	91.4
Rat Plasma (EDTA) (Wistar) (x4)	104.51	100.95	96.6
	67.01	65.98	98.5
	48.26	45.70	94.7

3. Intra - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
133.20	3.88	2.9	24
35.34	1.66	4.7	24
8.17	0.42	5.1	24

4. Inter - Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
136.46	6.00	4.4	44
34.23	1.81	5.3	44
7.75	0.59	7.6	44

5. Specificity

Compound	Cross Reactivity
Rat GRO/CINC-1	100.0%
Rat GRO/CINC-2 α	$\leq 0.1\%$
Rat GRO/CINC-2 β	$\leq 0.1\%$
Rat GRO/CINC-3	$\leq 0.1\%$
Mouse GRO β /MIP-2	$\leq 0.1\%$

6. Sensitivity

0.35 pg/mL

The sensitivity for this kit was determined using the guidelines under the National Committee for Clinical Laboratory Standards (NCCLS) Evaluation Protocols. (National Committee for Clinical Laboratory Standards Evaluation Protocols, SC1, (1989) Villanova, PA: NCCLS.

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2~8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to contact your skin and clothes with "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. The precipitation may grow in "2, Labeled antibody Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from different lot or different kit.
8. Do not use the reagents expired.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8 °C
The expiry date is specified on outer box.

REFERENCES

1. Watanabe K, Konishi K, Fujioka M, Kinoshita S, Nakagawa H. The Neutrophil Chemoattractant Produced by the Rat Kidney Epithelioid Cell Line NRK-52E Is a Protein Related to the KC/gro Protein. J Biol Chem., 1989 : 264,19559-19563.
2. Yamasaki Y, Matsuo Y, Zagorski J, Matsuura N, Onodera H, Itoyama Y, Kogure K. New therapeutic possibility of blocking cytokine-induced neutrophil chemoattractant on transient ischemic brain damage in rats. Brain Research., 1997 : 759,103-111.
3. Hashimoto S, Pittet JF, Hong K, Folkesson H, Bagby G, Kobzik L, Frevert C, Watanabe K, Tsurufuji S, Wiener-Kronish J. Depletion of alveolar macrophages decreases neutrophil chemotaxis to Pseudomonas airspace infections. Am.J.of Physiol., 1996 : 270, L819-L828.
4. Tsuruma T, Yagihashi A, Tarumi K, Hirata K. Anti-rat IL-8 (CINC) monoclonal antibody administration reduces ischemia-reperfusion injury in small intestine. Transplant Proc., 1998 : 30,2644-2645.
5. Effect of anti-macrophage migration inhibitory factor antibody on lipopolysaccharide-induced pulmonary neutrophil accumulation. Makita H, Nishimura M, Miyamoto K, Nakano T, Tanino Y, Hirokawa J, Nishihira J, Kawakami Y. Am J Respir Crit Care med., 1998 : 158, 573-579.

Version 1.