Code No. 17176

Rat MCP-1 Assay Kit - IBL

INTRODUCTION

MCP-1 (Monocyote Chemoattractant protein-1) is the basic protein consisting of 76 amino acids
and is classified as C-C sub family of chemokines. MCP-1 shows specific chemotaxis to the monocyote and relates to the manifestation of desmosomal molecule on the surface of Monocyte, the Monocyte chemotaxis to the inflammation site, conjugation with endothelial cell and to the exudation to sub-endothelium. It is produced in chronic inflammatory diseases such as chronic arthritis and nephritis and in malignant tumors such as atherosclerosis and others and is thought to have important role to the regional exudation of Monocyte and macrophage.

PRINCIPLE

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

MEASUREMENT RANGE

50 ~ 3,200 pg/mL

INTENDED USE

- This ELISA kit is a complete kit for the quantitative determination of Rat MCP-1 in serum, urine and supernatant of cell culture media.
- Both recombinant and native forms of Rat MCP-1 can be detected with the kit.

KIT COMPONENT

1	Precoated plate	: Anti- Rat MCP-1 Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody	: HRP conjugated Anti-Rat MCP-1 Mouse IgG Fab' Affinity Purify	10.5mL x 1
3	EIA buffer	: 1% BSA, 0.05% Tween 20 in PBS	30mL x 1
4	Standard	: Recombinant Rat MCP-1	0.5mL x 1
5	Missing number (It becomes missing number because kit component has been changed.)		
6	Stop solution	: 1N H_2SO_4	11mL x 1
7	Chromogen	: TMB solution	15mL x 1
8	Wash buffer Conc.	: (40X)0.05% Tween20 in phosphate buffer	50mL x 1
9	Solution for Labeled antibody	: 1% BSA, 0.05%Tween20 in PBS	10.5mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Micropipette and tip
- Graduated cylinder and beaker
- Deionized water
- Incubator (37℃±1℃)
- Graph paper (log/log)
- Paper towel
- Tube for dilution of Standard
- Washing bottle for precoated plate
- Disposable test tube for "7, Chromogen"

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.

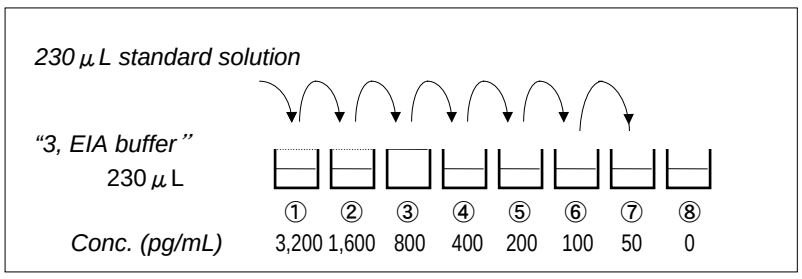
2) Solution of labeled antibody
Put 10.5mL of "9, Solution for Labeled antibody" into the vial of "2, Labeled antibody" and place it at room temperature for 5 minutes. Then mix it gently and completely.
This operation should be done <u>just before the application of labeled antibody.</u>

3) Preparation of Standard
Put just 0.5mL of deionized water into the vial of "4, Standard" and mix it gently and completely. This solution is 6,400pg/mL Rat MCP-1 standard.

4) Dilution of Standard
Prepare 8 tubes for dilution of "4, Standard". Put 230μL each of "3, EIA buffer" into the tube.
Specify the following concentration of each tube.

Tube-1	3,200 pg/mL
Tube-2	1,600 pg/mL
Tube-3	800 pg/mL
Tube-4	400 pg/mL
Tube-5	200 pg/mL
Tube-6	100 pg/mL
Tube-7	50 pg/mL
Tube-8	0 pg/mL (Test Sample Blank)

Put 230μL of Standard solution into tube–1 and mix it gently. Then, put 230 μL of tube-1mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 3200 pg/mL and 50 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 "3, EIA buffer" as the need arises.
If the concentration of Rat MCP-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 100μL	Diluted standard (Tube 1~7) 100μL	EIA buffer (Tube-8) 100μL	EIA buffer 100μL
Incubation for 1 hour at 37℃ with plate lid				
Washing 7 times				
Labeled Antibody	100μL	100μL	100μL	-
Incubation for 30minutes at 37℃ with plate lid				
Washing 9 times				
Chromogen	100μL	100μL	100μL	100μL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100μL	100μL	100μL	100μL
Read the plate at 450nm agaomst a Reagent Blank within 30 minutes after addition of Stop solution.				

1) Determine wells for reagent blank. Put 100μL each of "3, EIA buffer" into the wells.
2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100μL 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℃ after covering it with plate lid.
4) Wash each well of the precoated plate vigorously with Wash buffer using the washing bottle. Then, fill each well with Wash buffer and leave the precoated plate lay for 15~30 seconds. Remove Wash buffer completely from the precoated plate by snapping.
<u>This procedure must be repeated more than 7 times.</u>
Then, remove the remaining liquid from all wells completely by snapping the precoated plate onto paper towel.
In case of using a plate washer, after 4 times washing with plate washer, washing with above washing bottle must be repeated 3 times.
5) Pipette 100μL 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℃ after covering it with plate lid.
7) Wash the precoated plate 9 times in the same manner as 4).
8) "7, Chromogen" should be taken the required quantity into a disposable test tube. Then, pipette 100μL from the test tube into the wells. Please do not return the rest of test tube to "7, Chromogen" bottle to avoid contamination.
9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by the addition of "7, Chromogen"
10) Pipette 100μL of "6, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by the addition of "6, 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 against a reagent blank.
The measurement shall be done within 30minutes after the addition of "6, Stop solution" .

SPECIAL ATTENTION

1. Test samples should be measured soon after collection. For the storage of test samples, store them frozen 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 "3, EIA buffer" , if the need arises.
3. Duplicate measurement of test samples and standard 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. "7, Chromogen" should be stored in the dark due to its sensitivity against light. "7, Chromogen" should be avoided contact with metals.
8. Measurement should be done within 30 minutes after addition of "6, Stop

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solution".

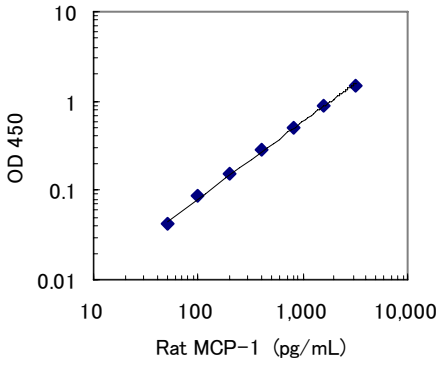
9. Storage of HRP conjugated antibody is not recommended. However, if the HRP conjugates do not use at one time, please store it at below -20℃.

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)
3,200	1.548
1,600	0.933
800	0.549
400	0.327
200	0.204
100	0.134
50	0.091
0 (Test Sample Blank)	0.049



＊ 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	1	3,117	3,200	97.4
	2	1,704	1,600	106.5
	4	849	800	106.1
	8	433	400	108.3
	16	193	200	96.5
	32	89	100	89.0
	64	47	50	94.0
Normal Rat Serum	8	854.4	1,005.8	84.9
	16	477.6	502.9	95.0
	32	247.1	251.5	98.3
	64	115.8	126.0	91.9
	128	61.3	62.8	97.6
Normal Rat Urine	2	2868.9	2966.4	96.7
	4	1594.5	1635.4	97.5
	8	907.6	804.5	112.8

2. Added Recovery Assay

Specimen	Theoretical Value (pg/mL)	Measurement Value (pg/mL)	%
10% FCS added RPMI-1640	3,200	2,869	89.7
	1,600	1,603	100.2
	800	799	99.9
	400	385	96.3
	200	194	97.0
	100	90	90.0
Rat Serum (X 2)	635.5	523.6	82.4
	435.3	364.5	83.7
	335.3	295.9	88.2
	285.3	282.8	99.1
Rat Urine (X 4)	940.5	966.8	102.8
	540.5	534.3	98.9
	340.5	339.0	99.6

3. Intra – Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
1919.2	85.4	4.4	8
455.3	16.4	3.6	8
83.6	2.9	3.5	8

4. Inter – Assay

Measurement Value (pg/mL)	SD value	CV value (%)	n
1767.2	80.8	4.6	24
449.6	26.6	5.9	24
82.9	4.3	5.2	24

5. Specificity

Compound	Cross Reactivity
Rat MCP-1	100.0%
Rat GRO/CINC-1	≦0.1%
Rat GRO/CINC-2α	≦0.1%
Rat GRO/CINC-2β	≦0.1%
Rat GRO/CINC-3	≦0.1%
Rat Rantes	≦0.1%
Rat MIP-1α	≦0.1%
Rat IL-1β	≦0.1%
Rat IL-6	≦0.1%
Rat Leptin	≦0.1%

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2-8℃. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. HRP conjugated antibody and standard are lyophilized products. Be careful to open these vials.
3. "6, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "6, Stop solution" and pay attention to the disposal of "6, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. Wash hands after handling reagents.
6. Do not mix the reagents with the reagents from a different lot or kit.
7. Do not use the expired reagents.
8. 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.

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