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Product Information

Rat MCP-1/CCL2 ELISA Kit
for cell and tissue lysates

Catalog Number **RAB0058**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

The Rat MCP-1 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of rat MCP-1 in cell lysate and tissue lysates. This assay employs an antibody specific for rat MCP-1 coated on a 96 well plate. Standards and samples are pipetted into the wells and MCP-1 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-rat MCP-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 MCP-1 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Components

1. Rat MCP-1/CCL2 Antibody-coated ELISA Plate (Item A) - RABRMCP1A: 96 wells (12 strips × 8 wells) coated with anti-rat MCP-1.
2. 20× Wash Buffer (Item B) - RABWASH4: 25 mL of 20× concentrated solution.
3. Lyophilized Rat MCP-1/CCL2 Protein Standard (Item C) - RABRMCP1S: 2 vials, recombinant rat MCP-1.
4. ELISA 5× Sample Diluent Buffer (Item D2) - RABDIL6: 10 mL of 5× concentrated buffer. For Standard/Sample (cell lysate/tissue lysate) diluent.
5. ELISA 5× Assay/Sample Diluent Buffer E (Item E2) - RABELADE: 15 mL of 5× concentrated buffer. For Detection Antibody (Item F) and HRP-Streptavidin concentrate (Item G) diluent.
6. Biotinylated Rat MCP-1/CCL2 Detection Antibody (Item F) - RABRMCP1F: 2 vials of biotinylated anti-rat MCP-1(each vial is enough to assay half microplate).
7. HRP-Streptavidin (Item G) - RABHRP5: 200 µL of 100× concentrated HRP-conjugated streptavidin.
8. ELISA Colorimetric TMB Reagent (HRP Substrate, Item H) - RABTMB3: 12 mL of 3,3′,5,5′-tetra-methylbenzidine (TMB) in buffered solution.
9. ELISA Stop Solution (Item I) - RABSTOP3: 8 mL of 0.2 M sulfuric acid.
10. 2× Cell Lysis Buffer (Item J) - RABLYSIS1: 5 mL of 2× cell lysate buffer.

Reagents and Equipment Required but Not Provided.

1. Microplate reader capable of measuring absorbance at 450 nm
2. Precision pipettes to deliver 2 µL to 1 mL volumes
3. Adjustable 1-25 mL pipettes for reagent preparation
4. 100 mL and 1 liter graduated cylinders
5. Absorbent paper
6. Distilled or deionized water
7. Log-log graph paper, or computer and software for ELISA data analysis
8. Tubes to prepare standard or sample dilutions

Precautions and Disclaimer

This product is for Research Use Only. Not for Use in Diagnostic Procedures. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

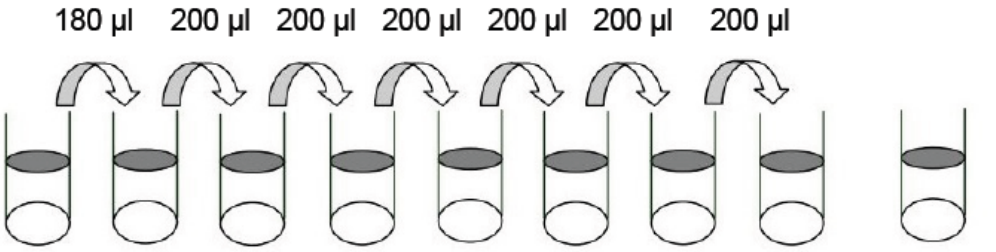
1. Bring all reagents and samples to room temperature (18–25 °C) before use.

2. Sample Diluent Buffer/Assay Diluent should be diluted 5-fold with deionized or distilled water. Cell lysate buffer (Item J) should be diluted 2-fold with deionized or distilled water (for cell lysate and tissue lysate).

3. Sample dilution: Tissue lysate and cell lysate samples should be diluted at least 5-fold with 1× Sample Diluent Buffer. Generally, a minimum of 1 mg of protein per 1 ml of original lysate solution is recommended. Addition of protease inhibitors (not included) to the lysis buffer prior to use is also recommended.
   Note: Levels of MCP-1 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator.

4. Preparation of standard: **Briefly spin the vial of Item C**. Add 400 µL of 1× Sample Diluent Buffer (Item D, Sample Diluent Buffer should be diluted 5-fold with deionized or distilled water before use) into Item C vial to prepare a 50 ng/mL standard. Dissolve the powder thoroughly by a gentle mix. Add 180 µL of MCP-1 standard from the vial of Item C into a tube with 320 µL of 1× Sample Diluent Buffer to prepare 18,000 pg/mL stock standard solution. Pipette 400 µL of 1× Sample Diluent Buffer into each tube. Use the stock standard solution to produce a dilution series (see Figure 1). Mix each tube thoroughly before the next transfer. 1× Sample Diluent Buffer serves as the zero standard (0 pg/mL).

Figure 1.
Dilution Series for Standards



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	320 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	50 ng/ml	18000 pg/ml	6000 pg/ml	2000 pg/ml	666.7 pg/ml	222.2 pg/ml	74.07 pg/ml	24.67 pg/ml		0 pg/ml

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

6. Briefly spin the Detection Antibody vial (Item F) before use. Add 100 µL of 1× Assay Diluent into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4 °C for 5 days). The detection antibody concentrate should be diluted 65-fold with 1× Assay Diluent and used in Procedure, step 5.

7. Briefly spin the HRP-Streptavidin concentrate vial (Item G) and pipette up and down to mix gently before use. HRP-Streptavidin concentrate should be diluted 100-fold with 1× Assay Diluent. For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 100 µL of HRP-Streptavidin concentrate into a tube with 10 mL of 1× Assay Diluent to prepare a final 100-fold diluted HRP-Streptavidin solution. Mix well.

Storage/Stability
Store the kit at –20 °C. It remains active for up to 1 year. Avoid repeated freeze-thaw cycles.

The reconstituted standard should be stored at –20 °C or –70 °C (–70 °C is recommended). Opened microplate strips or reagents may be store for up to 1 month at 2–8 °C. Return unused wells to the pouch containing desiccant pack and reseal along entire edge.

Procedure
1. Bring all reagents and samples to room temperature (18–25 °C) before use. It is recommended that all standards and samples be run at least in duplicate.

2. Label removable 8 well strips as appropriate for the experiment.

3. Add 100 µL of each standard (see Preparation Instructions, step 4) 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.

4. Discard the solution and wash 4 times with 1× Wash Solution. Wash by filling each well with 1× Wash Buffer (300 µL) using a multichannel 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.

5. Add 100 µL of 1× prepared biotinylated antibody (Preparation Instructions, step 6) to each well. Incubate for 1 hour at room temperature with gentle shaking.

6. Discard the solution. Repeat the wash as in step 4.

7. Add 100 µL of prepared Streptavidin solution (see Preparation Instructions, step 7) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

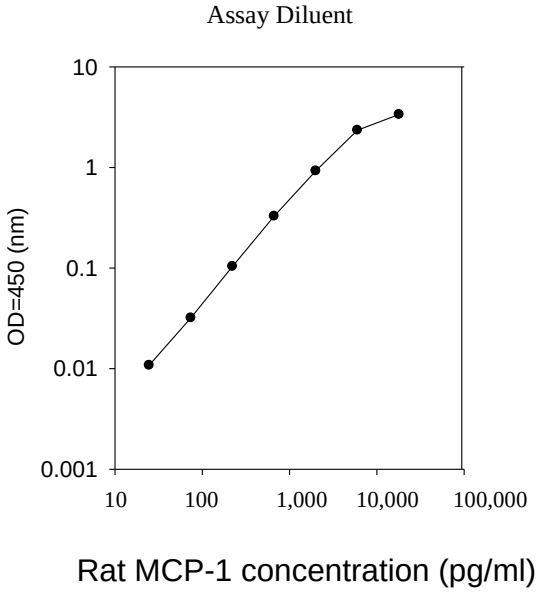
8. Discard the solution. Repeat the wash as in step 4.

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

10. Add 50 µL of Stop Solution (Item I) to each well. Read at 450 nm immediately.

Results
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 or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Typical Data
Standard curve(s) is for demonstration only. A Standard curve(s) must be run with each assay.



Product Profile

Sensitivity: The minimum detectable dose of rat MCP-1 was determined to be 15 pg/ml.

Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluent buffer).

Reproducibility:

Intra-Assay: CV <10%

Inter-Assay: CV <12%

Spiking & Recovery: Recovery was determined by spiking various levels of rat MCP-1 into rat cell lysate and tissue lysate. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Tissue lysate	92.75	83-103
Cell lysate	93.73	84-105

Linearity:

Sample Type		Tissue Lysate	Cell lysate
1:2	Average % of	92	93
	Expected Range (%)	83-103	84-104
1:4	Average % of	93	94
	Expected Range (%)	84-104	85-105

Specificity

The antibody pair provided in this kit recognizes rat MCP-1/CCL2.

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested *(e.g.*, rat CINC-2, CINC-3, CNTF, IL-1α, IL-1β, IL-4, IL-6, IL-10, GM-CSF, IFN-γ, Leptin, Lix, MIP-3α, β-NGF, TIMP-1, TNF-α, and VEGF).

Appendix
Troubleshooting Guide

Problem	Cause	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standard dilution	Ensure a brief spin of Item C and dissolve the powder thoroughly with gentle mixing.
Low signal	Too brief incubation times	Ensure sufficient incubation time; Procedure, step 3 may be done over night at 4 °C with gentle shaking. Note: may increase overall signals including background.
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
	Improper preparation of standard and/or biotinylated antibody	Briefly spin down vials before opening. Dissolve the powder thoroughly.
Large CV	Inaccurate pipetting	Check pipettes
	Air bubbles in wells	Remove bubbles in wells
High background	Plate is insufficiently washed	Review the manual for proper wash. If using a plate washer, ensure that all ports are unobstructed.
	Contaminated wash buffer	Make fresh wash buffer
Low sensitivity	Improper storage of the ELISA kit	Store the standard at <–20 °C after reconstitution, others at 4 °C. Keep substrate solution protected from light
	Stop solution	Add stop solution to each well before reading plate

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