BioLegend®

Enabling Legendary Discovery™

# Mouse CCL8 (MCP-2)

## ELISA MAX™ *Deluxe Set*

**Cat. No. 446904**

BioLegend's ELISA MAX™ Deluxe Sets contain the components necessary for the accurate quantification of natural and recombinant mouse CCL8 (MCP-2). These sets are designed for cost-effective and accurate quantification of mouse CCL8 (MCP-2) in cell culture supernatant, serum, plasma or other biological fluids. They are sensitive, accurate, and robust.

***It is highly recommended that this instruction sheet be read in its entirety before using this product. Do not use this set beyond the expiration date.***

## Materials Provided

1. Mouse CCL8 (MCP-2) ELISA MAX™ Capture Antibody (200X)
2. Mouse CCL8 (MCP-2) ELISA MAX™ Detection Antibody (200X)
3. Mouse CCL8 (MCP-2) Standard
4. Avidin-HRP (1000X)
5. Substrate Solution C
6. Coating Buffer B (5X)
7. Assay Diluent A (5X)

## Introduction

CCL8, also known as MCP-2, is a CC chemokine of the monocyte chemoattractant protein (MCP) family that plays key roles in allergic and inflammatory responses. CCL8 is constitutively expressed in the skin and lymph nodes of mice. Mouse CCL8 induces a chemotactic migration of a population of differentiated Th2 cells enriched in IL-5 and IL-25 receptors by binding to the CCR8 receptor. Recent studies have established CCL8 as a critical ligand for driving CCR8-dependent Th2 cell trafficking into the skin, which in conjunction with locally recruited eosinophils drives chronic cutaneous allergic inflammation. CCL8 expression by suprabasal buldge cells has also been experimentally shown to negatively regulate the population of mouse hair follicles by Langerhans cells, further underscoring this chemokines central contributions to maintaining cutaneous immunity.

## Principle of the Test

BioLegend ELISA MAX™ Deluxe Set contains pre-optimized essential reagents and additional buffers and solutions for Sandwich ELISA assay. A rat monoclonal anti-mouse CCL8 (MCP-2) antibody is first coated on a 96-well plate. Standards and samples are added to the wells, and CCL8 (MCP-2) binds to the immobilized capture antibody. Next, a biotinylated goat polyclonal anti-mouse CCL8 (MCP-2) detection antibody is added, producing an antibody-antigen-antibody "sandwich". The Avidin-HRP reagent is subsequently added, followed by TMB Substrate, producing a blue color in proportion to the concentration of CCL8 (MCP-2) present in wells. Then Stop Solution should be added to wells to terminate the reaction. This step changes the reaction color from blue to yellow. The absorbance in wells should be read at 450nm using a microplate reader.

***For research purposes only. Not for use in diagnostic or therapeutic procedures.***



## Materials to be Provided by the End-User

- Microwell plates: BioLegend Cat. No. 423501 is recommended
- Wash Buffer: BioLegend Cat. No. 421601 is recommended, or PBS + 0.05% Tween-20
- Stop Solution: BioLegend Cat. No. 423001 is recommended, or acid solution, e.g. 2N $H_2SO_4$
- Plate Sealers: BioLegend Cat. No. 423601 is recommended
- PBS (Phosphate-Buffered Saline): 8.0 g NaCl, 1.16 g $Na_2HPO_4$, 0.2 g $KH_2PO_4$, 0.2 g KCl, add deionized water to 1 L; pH to 7.4, 0.2 µm filtered
- Deionized (DI) water
- A microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 2 µL to 1 mL
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer

## Storage Information

- Store kit components between 2°C and 8°C.
- After reconstitution of the lyophilized standard with 1X Assay Diluent A, aliquot into polypropylene vials and store at -70°C for up to one month. Avoid repeated freeze/thaw cycles.
- Prior to use, bring all components to room temperature (18°C-25°C). Upon assay completion, return all components to appropriate storage conditions.

## Health Hazard Warnings

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details (www.biolegend.com/msds).
2. Substrate Solution C is harmful if ingested. Additionally, avoid skin, eye or clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

## Specimen Collection and Handling

***Cell Culture Supernatant:*** If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at < -20°C. Avoid repeated freeze/thaw cycles.

***Serum:*** Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 X *g*. Remove serum layer and assay immediately or store serum samples at < -20°C. Avoid repeated freeze/thaw cycles. Serum specimens should be clear and non-hemolyzed.

***Plasma:*** Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 20 minutes at 1,000 x g within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

## Reagent and Sample Preparation

***Do not mix reagents from different sets or lots. Reagents and/or antibodies from different manufacturers should not be used with this set. All reagents should be diluted immediately prior to use.***

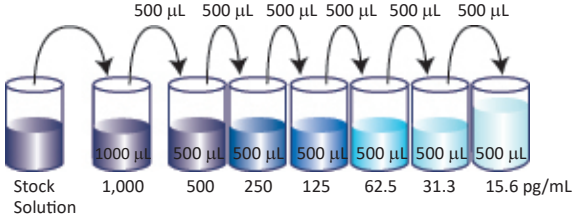
**NOTE: Precipitation of 5X Assay Diluent A may be observed when stored long term between 2°C and 8°C. The precipitation does not alter the performance of the Buffer. If heavy precipitation is observed after the dilution to 1X Assay Diluent A, it can be filtered to clarify the solution.**

**Preparation of 1X Reagent for 1 Plate**

| Material | Dilute with |
|---|---|
| 2.4 mL of Coating Buffer B (5X) | 9.6 mL of Deionized Water |
| 60 µL of Capture Antibody (200X) | 12 mL of 1X Coating Buffer B |
| 12 mL of Assay Diluent A (5X) | 48 mL of PBS |
| 60 µL of Detection Antibody (200X) | 12 mL of 1X Assay Diluent A |
| 12 µL of Avidin-HRP (1,000X) | 12 mL of 1X Assay Diluent A |

Lyophilized vials are under vacuum pressure. Reconstitute lyophilized standard by following the instructions described in Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol. Allow the reconstituted standard to sit for 15 minutes at room temperature, then mix gently prior to making dilutions.

Prior to use, prepare 1000 µL of the top standard at a concentration of 1,000 pg/mL from the stock solution in 1X Assay Diluent A (**refer to Lot-Specific Certificate of Analysis/ELISA MAX™ Deluxe Set Protocol**). Perform six two-fold serial dilutions of the 1,000 pg/mL top standard with 1X Assay Diluent A in separate tubes. After diluting, the mouse CCL8 (MCP-2) standard concentrations are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL, and 15.6 pg/mL, respectively. 1X Assay Diluent A serves as the zero standard (0 pg/mL).




Samples: In general, serum and plasma samples require at least 400-fold dilution. For cell culture supernatant samples, CCL8 (MCP-2) can vary from sample to sample. If dilution is required, use 1X Assay Diluent A as the sample diluent.

## Assay Procedure

***Do not use sodium azide in any solutions as it inhibits the activity of the horseradish-peroxidase enzyme.***

1. One day prior to running the ELISA, dilute Capture Antibody in 1X Coating Buffer B as described in Reagent Preparation. Add 100 µL/well of this Capture Antibody solution to the 96-well plate provided in this set. Seal plate and incubate overnight between 2°C and 8°C.
2. Bring all reagents to room temperature (RT) prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
3. Wash plate 4 times with at least 300 µL Wash Buffer per well and blot residual buffer by firmly tapping plate upside down on absorbent paper. **All subsequent washes should be performed similarly.**
4. To block non-specific binding and reduce background, add 200 µL 1X Assay Diluent A per well.
5. Seal plate and incubate at RT for 1 hour with shaking on a plate shaker (e.g. 500 rpm with a 0.3 cm circular orbit). All subsequent incubation with shaking should be performed similarly.

6. While plate is being blocked, prepare the appropriate sample dilutions (if necessary) and standards.
7. Wash plate 4 times with Wash Buffer.
8. Add 100 µL of standard dilutions or samples to the appropriate wells.
9. Seal plate, incubate at RT for 2 hours with shaking
10. Wash plate 4 times with Wash Buffer.
11. Add 100 µL/well of diluted Detection Antibody solution, seal plate and incubate at RT for 1 hour with shaking.
12. Wash plate 4 times with Wash Buffer.
13. Add 100 µL/well of diluted Avidin-HRP solution, seal plate and incubate at RT for 30 minutes with shaking.
14. Wash plate 5 times with Wash Buffer. For this final wash, soak wells in Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
15. Add 100 µL/well Substrate Solution C and incubate **in the dark** for 10 minutes. Positive wells should turn blue in color. It is not necessary to seal the plate during this step.
16. Stop reaction by adding 100 µL/well of Stop Solution. Positive wells should turn from blue to yellow.
17. Read absorbance at 450 nm within 15 minutes. If the reader can read at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

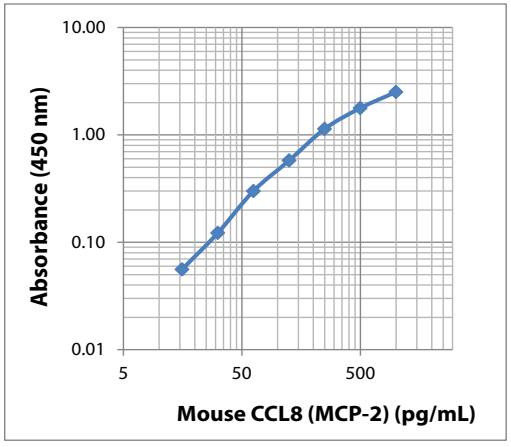
***Optimal substrate incubation time depends on laboratory conditions and the optical linear ranges of ELISA plate readers.**

## Calculation of Results

The data is best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If the samples were diluted, multiply by the appropriate dilution factor. If a test sample's absorbance value falls outside the standard curve ranges, that test sample needs to be reanalyzed at a higher or lower dilution as appropriate.

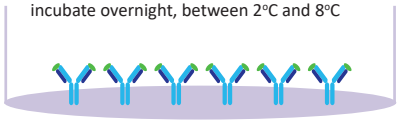
## Typical Data

***Standard Curve:*** This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.

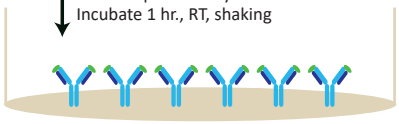



## Assay Procedure Summary

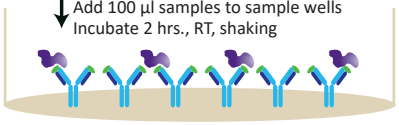
1. Coat plate with 100 µL diluted Capture Antibody incubate overnight, between 2°C and 8°C



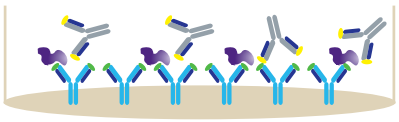
2. Wash 4 times
   Add 200 µL 1X Assay Diluent A
   Incubate 1 hr., RT, shaking



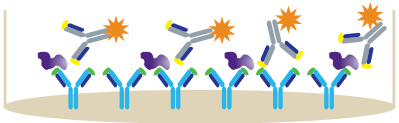
3. Add 100 µl diluted standards to standard wells
   Add 100 µl samples to sample wells
   Incubate 2 hrs., RT, shaking



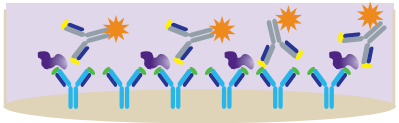
4. Wash 4 times
   Add 100 µL diluted Detection Antibody
   Incubate 1 hr., RT, shaking



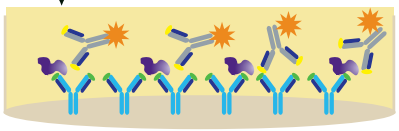
5. Wash 4 times
   Add 100 µL Avidin-HRP
   Incubate 30 min. RT, shaking



6. Wash 5 times
   Add 100 µL Substrate Solution C
   Incubate 10 min. RT, in the dark



7. Add 100 µL Stop Solution



8. Read absorbance at 450nm and 570nm

## Performance Characteristics

***Sensitivity:*** The expected minimum detectable concentration of human for this set is 5.1 ± 3.3 pg/mL.

***Specificity:*** No cross-reactivity was observed when this kit was used to analyze multiple mouse and human recombinant proteins, each at 50 ng/mL.

## Troubleshooting

High Background:

- Background wells were contaminated.
- Matrix used had endogenous analyte.
- Plate was insufficiently washed.
- TMB Substrate Solution was contaminated.

No signal:

- Incorrect or no antibodies were added.
- Avidin-HRP was not added.
- Substrate solution was not added.
- Wash buffer contains sodium azide.

Low or poor signal for the standard curve:

- Standard was incompletely reconstituted or was stored improperly.
- Reagents were added to wells with incorrect concentrations.
- Plate was incubated with improper temperature, timing or agitation.

Signal too high, standard curves saturated:

- Standard was reconstituted with less volume than required.
- One or more reagent incubation steps were too long.
- Plate was incubated with inappropriate temperature, timing, or agitation.

Sample readings out of range:

- Samples contain no or below detectable levels of analyte.
- Samples contain analyte concentrations greater than highest standard point.

High variations in samples and/or standards:

- Pipetting errors may have occurred.
- Plate washing was inadequate or non-uniform.
- Samples were not homogenous.
- Samples or standard wells were contaminated.

**BioLegend, Inc.**

**BioLegend is ISO 13485 Certified**

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