OmniKine™

Murine MDC

Chemiluminescent Sandwich ELISA Kit

Catalog #: Lum-8193

Detection and Quantification of Murine MDC Concentrations in Supernatants, Sera and Plasma.

Research Purposes Only. Not Intended for Diagnostic or Clinical Procedures.

Store entire kit at 4°C until use.
Kit expiration is 3 months from date of shipment

Manual Version: 1.8.622

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INTRODUCTION

Murine MDC, also known as mouse macrophage-derived chemokine, ABCD-1 or CCL22, is a 7.8 kDa signaling protein produced in B lymphocytes (B-cells), microglia, monocyte-derived dendritic cells, activated cytotoxic lymphocytes (NK, natural killer cells), CD4 T lymphocytes (T-cells) and many more cell types. This protein, expressed from the gene Ccl22, is known to be a member of the C-C or β-chemokine family through its intrinsic pair of disulfide linkages formed by four conserved cysteine residues. In addition, the protein also consists of a C-terminal heparin-binding motif and no potential N-linked glycosylation sites. The precursor mMDC protein is initially synthesized as a 92 amino acid polypeptide characterized by an N-terminal signal sequence followed by the actual mMDC polypeptide. After proteolytic cleavage of the 24 amino acid residue signal sequence, the 68 residue mMDC peptide is allowed to fold into a mature mMDC protein where it can provide support to a plethora of immune functions. Normally, chemokines undergo significant N-terminal processing which allows for up- and down-regulation of their physiology and function. Moreover, some chemokines are subject to processing by peptidases, considerably reducing their activity on neighboring lymphocytes and monocyte-derived dendritic cells. The MDC protein is implicated in a variety of pathways regarding lymphocyte migration. Through its high affinity binding with CCR4 receptors studded on the plasma membranes of activated T cells, the MDCs expressed by dendritic cells are able to chemoattract these lymphocytes to augment immune functions. In addition, MDCs expressed by dendritic cells have high affinity for and can chemically attract CD4+, CD25+ and CTLA4+ regulatory T cells – all displaying membrane CCR4 receptors – to inflammatory regions where T cells have weakened activation. Another type of MDC produced from B lymphocytes can help stimulate B-T cell interactions through the formation of germinal centers, or sites within lymph nodes that provide a hub for B-cell proliferation, differentiation and hyper-mutation through activation by T-cell dependent antigens. Furthermore, countless studies have revealed that the MDC protein can function as a powerful chemoattractant for dendritic cells, hematopoietic precursor cells and activated cytotoxic killer cells, as well as activate platelet functions and inhibit hippocampal synapses.

ASSAY PRINCIPLES

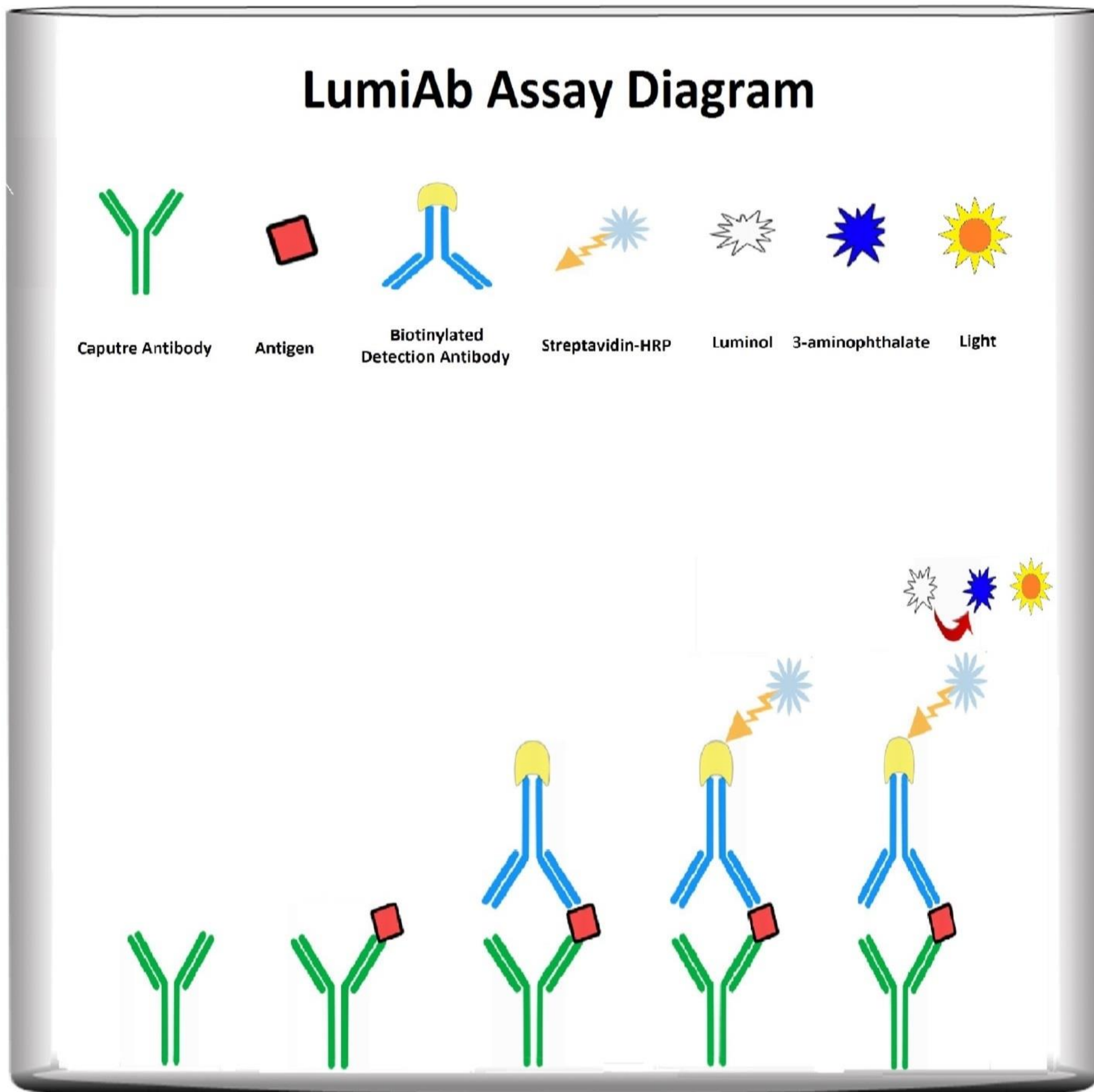
The LumiAb™ Murine MDC ELISA Kit contains the components necessary for quantitative determination of natural or recombinant concentrations within any experimental sample including cell lysates, serum and plasma. This particular immunoassay utilizes the quantitative technique of a “Sandwich” Enzyme-Linked Immunosorbent Assay (ELISA) where the target protein (antigen) is bound in a “sandwich” format by the primary capture antibodies coated to each well-bottom and the secondary detection antibodies added subsequently by the investigator.

The capture antibodies coated to the bottom of each well are specific for a particular epitope on while the user-added detection antibodies bind to epitopes on the captured target protein. Amid each step of the procedure, a series of wash steps must be performed to ensure the elimination of non-specific binding between proteins to other proteins or to the solid phase.

After incubation and “sandwiching” of the target antigen, a peroxidase enzyme is conjugated to the constant heavy chain of the secondary antibody (either covalently or via Avidin/Streptavidin-Biotin interactions), allowing for a sensitive luminescent reaction to ensue upon substrate addition.

When the Peroxide Enhancer solution is added, the reaction catalyzed by peroxidase yields light that is representative of the antigen concentration. After a brief incubation, the microplate can be read with a luminometer, allowing for generation of a standard curve and subsequent determination of protein concentration.

ASSAY FORMAT



① Capture anitbodies are coated onto microplate

② Sample is added and any antigen present is bound by the capture antibody

③ Biotinylated detection antibody is introduced, sandwiching the target

④ Streptavidin-HRP binds to biotin via high affinity interaction

⑤ Luminol is catalyzed by HRP and emission of light is read by the luminometer

MATERIALS INCLUDED

<table>
<tr><th>Component</th><th>Quantity Per Plate</th><th>Storage/Stability after first use</th></tr>
<tr><td>Microstrips Coated w/ Capture Antibody</td><td>12 x 8-Well Microstrips</td><td rowspan="8">1 month at 4°C</td></tr>
<tr><td>Protein Standard</td><td>Lyophilized</td></tr>
<tr><td>100^{x} Biotinylated Detection Antibody</td><td>Lyophilized</td></tr>
<tr><td>400^{x} Streptavidin-HRP</td><td>30 µl</td></tr>
<tr><td>Wash Buffer (15^{x})</td><td>50 ml</td></tr>
<tr><td>Assay Diluent 1TD</td><td>50 ml</td></tr>
<tr><td>Enhancer Solution</td><td>8 ml</td></tr>
<tr><td>Peroxide Solution</td><td>8 ml</td></tr>
<tr><td>Adhesive Plate Sealers</td><td>2 Sheets</td><td>-</td></tr>
<tr><td>Technical Manual</td><td>1 Manual</td><td>-</td></tr>
</table>

Any unused strips should be rewrapped with plate sealer and placed back into pouch with zipper closed until next use**

ADDITIONAL MATERIALS REQUIRED

The following materials and/or equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:

- Luminometer able to measure total light output
- Micropipettes ranging from 1 µl to 1 ml
- Distilled, deionized, and or purified water (recommended TOC 1-50 ppb, MΩ-cm 18.0)
- Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer
- Graph paper or computer software capable of generating or displaying logarithmic functions
- Absorbent paper or vacuum aspirator
- Test tubes or microfuge tubes capable of storing ≥1 ml
- Bench-top centrifuge (optional)
- Bench-top vortex (optional)
- Orbital shaker (optional)

SAMPLE PREPARATION AND STORAGE

Levels of Murine MDC may vary between samples. Optimal dilution factors for every sample must be determined by the investigator.

Cell Supernatants

Remove large cell components via centrifugation and perform the assay. Cell lysates and supernatants require a dilution using Assay Diluent 1TD. A serial dilution may be performed to determine a suitable dilution factor for the sample.

Serum

Allow samples to clot in a serum separator tube (SST) for 30 minutes. After sufficient clotting, centrifuge at 1000 x g for 15 minutes and remove serum from SST in preparation for the assay. A serial dilution may be performed to determine a suitable dilution factor for the sample. For serum sample dilutions refer to Serum and Plasma Sample Dilution Protocol.

Plasma

Use heparin, citrate or EDTA as an anticoagulant to gather plasma from original biological sample. After collection of the plasma, centrifuge for 15 minutes at 1000 x g. This step must be performed within 30 minutes of plasma collection. A serial dilution may be performed to determine a suitable dilution factor for the sample. For plasma sample dilutions refer to Serum and Plasma Sample Dilution Protocol.

If samples are to be used within 24 hours, aliquot and store at 4°C. If samples are to be used over a long period of time, aliquot and store between -20°C and -80°C, depending on the duration of storage.

Samples containing a visible precipitate or pellet must be clarified prior to use in the assay.

Avoid repeated freeze/thaw cycles to prevent loss of biological activity of proteins in experimental samples.

Serum and Plasma Sample Dilution Protocol

a. Dilute the serum or plasma samples with PBS supplemented with 10-50% animal serum (Serum/Plasma Diluent).

b. Reconstitute and dilute the Protein Standards using the Serum/Plasma Diluent, instead of Assay Diluent 1TD, so it reflects the environment of the samples being measured.

c. Reconstitute the Biotin-Conjugated Detection Antibody in Assay Diluent 1TD and dilute the Streptavidin-HRP in Assay Diluent 1TD. Do not use the Serum/Plasma Diluent to reconstitute or dilute the Detection Antibody or Streptavidin-HRP.

REAGENT PREPARATION

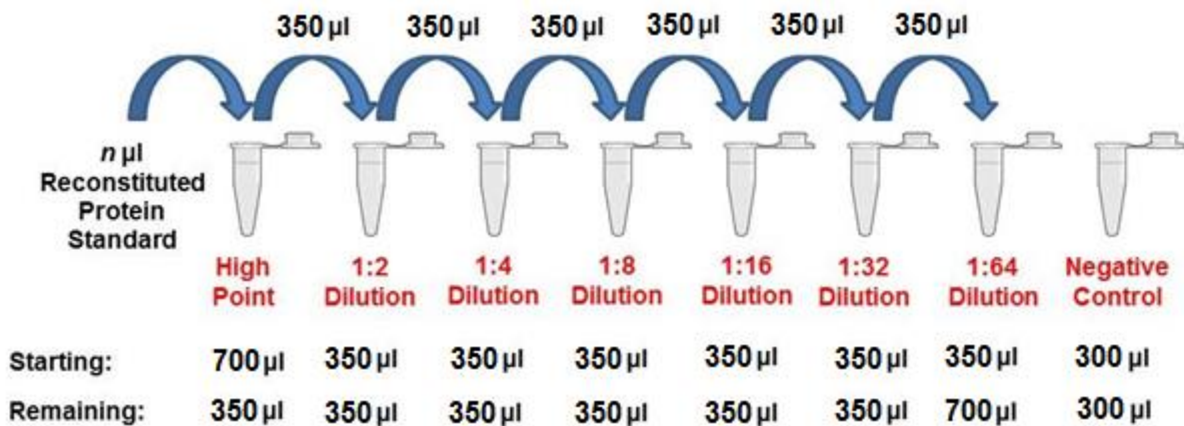
All provided solutions should be at ambient temperature prior to use. **We recommend performing the assay in duplicate**. Reagents provided are enough to assay 96 wells and it is recommended to only prepare as much needed on the day of the experiment. All incubation steps should be performed on an orbital shaker to equilibrate solutions when added to the microplate wells.

1. Dilute the 15x Wash Buffer to 1x Wash Buffer using 14 volumes of ddH2O and 1 volume of 15x Wash Buffer.

2. Reconstitute Detection Antibody with 100 µl of ddH2O for a concentration of 100x. Mix gently and dilute to 1x prior to use.

3. Reconstitute Protein Standard with 83 µl of ddH2O for a concentration of 250 ng/mL Mix gently and dilute to the working range of the kit, 4-250 pg/mL.

    a. Dilute Protein Standard with the same reagents used with Serum/Plasma samples. **OR**.
    b. Dilute Protein Standard with Assay Diluent 1TD when experimenting with Cell Supernatant samples.



4. Mix the 400x Streptavidin-HRP (SAV-HRP) gently. Dilute to 1x using Assay Diluent 1TD.

5. Enhancer Solution and Peroxidase Solution are ready to use and do not need dilution. Mix at a 1:1 ratio to prior to incubation.

ASSAY PROCEDURE

1. Prepare all reagents to working concentrations, standards to desired range, and samples to appropriate dilution factors.

2. Remove desired number of capture antibody coated strips for experiment and place remaining strips back into dry pouch with desiccant for 4°C storage.

3. Add 100 µl of Standards/ Samples to each well and incubate on orbital shaker at room temperature (RT) for 2 hrs.

4. Aspirate the solution and add 300 µl of 1x Wash Buffer to each well being used and gently shake for 2-3 mins on an orbital shaker. Repeat this process 3 times. After the last wash ensure no liquid remains by inverting the plate and tapping it against clean paper towels.

5. Add 100 µl of 1x Detection Antibody to each well and incubate for 2 hrs. on an orbital shaker at RT

6. Repeat step 4.

7. Add 100 µl of 1x SAV-HRP to each well and incubate for 30 mins on an orbital shaker at RT

8. Repeat step 4.

9. Add 100 µl of combined Enhancer/Peroxide solution to each well, cover plate from light, and incubate for 5 mins on an orbital shaker at RT.

10. Read plate on Luminometer.

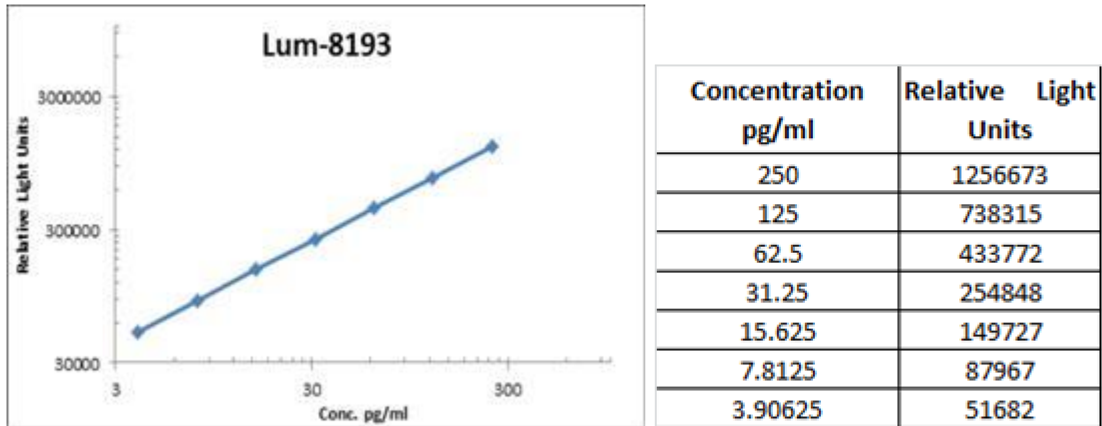
DATA ANALYSIS

Average the duplicate or triplicate readings for each standard, control and sample and subtract the average zero standard total light output (relative light units).

Generate a standard curve by using Microsoft Excel or other computer software capable of establishing a 4-Parameter Logistic (4-PL) curve fit. If using Excel or an alternative graphing tool, plot the average optical density values in absorbance units (y-axis) against the known standard concentrations in pg/ml (x-axis).

Only use the values in which a noticeable gradient can be established. Afterwards, generate a best fit curve or “trend-line” through the plotted points via regression analysis.

TYPICAL DATA



Concentration pg/ml	Relative Light Units
250	1256673
125	738315
62.5	433772
31.25	254848
15.625	149727
7.8125	87967
3.90625	51682

The data and subsequent graph were obtained after performing a sandwich ELISA for Murine MDC. Each known sample concentration was assayed in triplicate.

The standard curves shown are for demonstration. A new curve must be generated for each experiment.

SENSITIVITY

The Murine MDC ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant proteins within the range of 4-250 pg/ml.

CROSS REACTIVITY AND SPECIFICITY

The LumiAb™ Murine MDC ELISA is capable of recognizing both recombinant and naturally produced Murine MDC proteins. The antigens listed below were tested at 50 ng/mL and did not exhibit significant cross reactivity or interference.
• Human: MDC (67 aa), MDC (69 aa)
• Murine: C-10, CTACK, Eotaxin, Eotaxin-2, Exodus-2, JE, KC, MCP-2, MCP-3, MCP-5, MIG, MIP-1alpha, MIP-1beta, MIP-1gamma, MIP-2, MIP-3, MIP-3beta, RANTES

SUMMARIZED PROTOCOL

Reconstitute Biotinylated Detection Antibody and Protein Standard and dilute the 15x Wash Buffer as specified.

Perform serial dilution of Protein Standard and prepare samples as desired. See sample preparation section for instructions to dilute serum and plasma samples.

Add 100ul of Protein Standard, sample or control to each well and incubate for 2 hours at room temperature.

Aspirate Protein Standards, samples or controls out and wash plate 4 times.

Dilute Biotinylated Detection Antibody as specified. Add 100ul to each well and incubate for 2 hours at room temperature.

Aspirate Biotinylated Detection Antibody out and wash plate 4 times.

Dilute 400x Streptavidin-HRP as specified. Add 100ul of 1x Streptavidin-HRP to each well and incubate at room temperature for 30 minutes.

Aspirate 1x Streptavidin-HRP out and wash plate 4 times.

Add 100ul of Ready-to-Use Substrate to each well and incubate at room temperature for color development.

Add 100ul of Stop Solution and read plate at 450nm.

HEALTH AND SAFETY PRECAUTIONS

- Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.

- Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

ASSAY RESTRICTIONS

- This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.

- Materials included in this kit should NOT be used past the expiration date on the kit label.

- Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.

- Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.

- The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of background noise cannot be fully excluded until all factors have been tested using the assay kit.

- Individual results may vary due to differences in technique, plasticware and water sources.

ELISA PLATE TEMPLATE

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
								8
								9
								10
								11
								12

TECHNICAL SUPPORT

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