



Human CCR-5 (C Chemokine Receptor type5) ELISA Mg™ Set ELISA Set for Accurate protein Quantitation

Introduction:

CD195, also known as CCR5, is a 45 kD G protein-coupled seven transmembrane CC-chemokine receptor. It binds to MIP-1 α , MIP-1 β , and RANTES and is expressed on a subset of T cells and monocytes. CD195 mediates an intracellular signal thought to induce differentiation and proliferation. CCR5 has also been shown to act as a co-receptor for R5 HIV-1 cell entry; modification of CCR5 by sulfation contributes to the efficiency of HIV-1 entry. Acts as a co-receptor with CD4 for HIV-1.

Intended Use:

Mgenex Biosciences's ELISA Mg™ Set is specifically designed for the accurate quantitation of the specific protein from cell culture supernatant, serum, plasma or other bodily fluids. It is ready-to-use, accurate, and sensitive.

Principle of the Test:

Mgenex Biosciences's ELISA Mg™ Set is a sandwich Enzyme-Linked Immunosorbent Assay (ELISA). A protein specific monoclonal antibody is first coated on a 96-well plate. Subsequently, standards and samples are added to the wells, and the protein binds to the immobilized capture antibody. Next, a biotinylated antiprotein detection antibody is added, followed by avidin-horseradish peroxidase, producing an antibody-antigen-antibody "sandwich". TMB substrate solution is then added, producing a blue color in proportion to the amount of target present in the sample. Finally, the Stop Solution changes the reaction color from blue to yellow, and the microwell absorbances are read at 450 nm.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. Samples should be run at a number of dilutions to ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge to remove debris prior to analysis. Samples can be stored at $< -20^{\circ}$ C. Avoid repeated freeze/thaw cycles.

Serum: Use a serum separator tube and allow clotting for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Remove serum layer and assay immediately or store serum samples at $< -20^{\circ}$ C. Avoid repeated freeze/thaw cycles.

Plasma: Collect blood sample in a citrate, heparin or EDTA containing tube. Centrifuge for 10 minutes at 1000 x g within 30 minutes of collection. Assay immediately or store plasma samples at $< -20^{\circ}$ C. Avoid repeated freeze/thaw cycles.

Reagents preparation and assay Procedure: (See Manual for details)

Performance Characteristics:

Sensitivity: The minimum detectable concentration of target is 15 pg/ml.

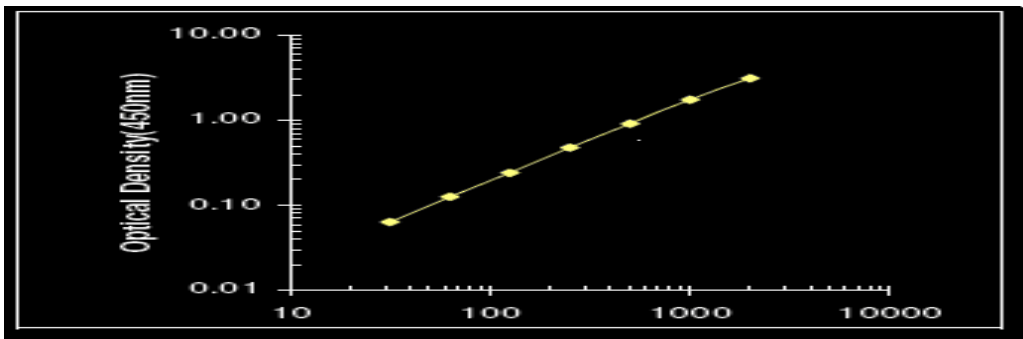
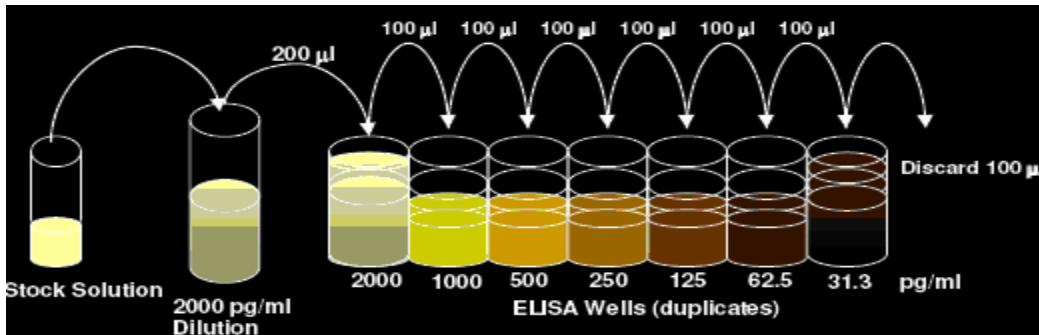
Recovery: 500 pg/ml was spiked into serum and plasma, and cell culture medium then analyzed by ELISA Mg™. At least 97% of the target was recovered from serum or plasma.

For cell culture supernatant, at least 93% target recovery was achieved.

Specificity: No cross reactivity was observed when this kit was used to analyze multiple human, mouse and rat recombinant proteins other than the specific target.

Typical Data:

This standard curve was generated at Mgenex Biosciences for demonstration purposes only. A standard curve must be run with each assay.

**Troubleshooting:****Poor Precision**

- _ Inadequate washing of wells
- _ Inadequate mixing of reagents
- _ Improper/inaccurate pipetting
- _ Improper plate sealing

Poor Signal

- _ Avoid sodium azide in wash buffers, as sodium azide inhibits the enzymatic activity of HRP.
- _ Verify that appropriate antibody pairs were used.
- _ Inadequate reagent volumes added to wells
- _ Incorrect incubation times and/or temperature

Poor Standard Curve

- _ Do not leave recombinant proteins at room temperature. If necessary, store recombinant proteins at 2-8°C for up to 8 hours prior to use.
- _ Recombinant protein vials should be quick-spun for maximum recovery.
- _ Incomplete washing of wells

High Background

- _ Increase stringency of washing steps by soaking plates for ~1 minute during washes prior to TMB addition.
- _ TMB substrate should be clear and colorless prior to addition to wells. Ensure that substrate was added to a clean container immediately prior to use.

Antigen References:

1. Samson, M., *et al.* 1996. *Biochemistry* 35:3362.
2. Raport, C.J., *et al.* 1996. *J. Biol. Chem.* 271:17161.
3. Combadiere, C., *et al.* 1996. *J. Leukoc. Biol.* 60:147.