



Rat MIP-1 α ELISA

Catalog Number EA-3008

(For Research Use Only)

Introduction

Macrophage Inflammatory Protein-1 (MIP-1 α) is a member of the C-C subfamily of chemokines that exhibit a variety of proinflammatory activities *in vitro* including leukocyte chemotaxis. MIP-1 is a major factor produced by macrophages after stimulated with bacterial endotoxins. It activates granulocytes (neutrophils, eosinophils and basophils) which can lead to acute neutrophilic inflammation. It also induces the synthesis and release of other pro-inflammatory cytokines such as IL-1, IL-6 and TNF α from fibroblasts and macrophages. In addition to its proinflammatory activities, MIP-1 α inhibits the proliferation of hematopoietic stem cells *in vitro* and *in vivo*.

Principle of the assay

MIP-1 α ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-rat MIP-1 α antibodies for immobilization on the microtiter wells and rabbit anti-rat MIP-1 α antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the MIP-1 α molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of MIP-1 α is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

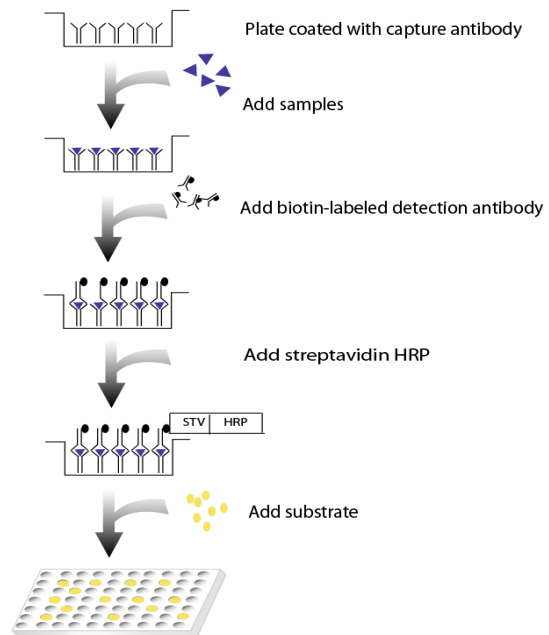


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with rabbit anti-rat MIP-1 α antibodies (4°C).
- Biotin labeled rabbit anti-rat MIP-1 α antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant Rat MIP-1 α standard (200ng/ml) (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (RT)
- Substrate (4°C).
- Stop Solution (4°C).

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

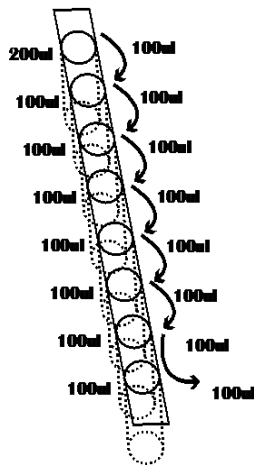
Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
40ml 5x Assay wash buffer
160ml ddH₂O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 100 times of Rat recombinant MIP-1 α (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions.
Add 2ul Rat Recombinant MIP-1 α in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled rabbit anti-rat MIP-1 α antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

7. Add 100 μ l of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100 μ l of substrate to each well and incubate for 10-30 minutes.
10. Add 50 μ l of Stop solution to each well. The color in the wells should change from blue to yellow.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. See instruction and diagram below for standard preparation.



- a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
- b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
- c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Add 100ul of sample per well and incubate for 1 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200 μ l of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100 μ l of diluted biotin-labeled mouse anti-rat MIP-1 α antibody to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4

Example of standard curve

