

Protein Standards for Mouse Inflammation ELISA Strip

Catalog Number EA-1052

(For Research Use Only)

100ul

| | | Stock | |
|-------------|--------------|----------|---------------|
| <u>Tube</u> | Protein | Conc. | <u>Volume</u> |
| #1 | Mouse IL-1a | 400ng/ml | 10ul |
| #2 | Mouse IL-1b | 400ng/ml | 10ul |
| #3 | Mouse G-CSF | 400ng/ml | 10ul |
| #4 | Mouse GM-CSF | 400ng/ml | 10ul |
| #5 | Mouse MCP-1 | 400ng/ml | 10ul |
| #6 | Mouse MIP-1a | 400ng/ml | 10ul |
| #7 | Mouse SCF | 400ng/ml | 10ul |
| #8 | Mouse Rantes | 400ng/ml | 10ul |
| | | | |

Preparation of protein standard dilutions

- 1. Add 200ul of Diluent buffer the wells of the first strip, and add 100ul of Diluent buffer to the wells of the rest strips according to the following table.
- 2. Add appropriate amount of protein standards to the first strip according to the table 1.
- 3. Use multi-channel pipette to mix the dilutions and transfer 100ul to the next dilution wells.
- 4. Repeat the transfer until 5th strip. Trash 100ul from 5th strip after mixing. Do not transfer to the 6th Strip

Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes. Because MIP-1a has a weaker signal than other cytokines' signals, it may require longer incubation time. Always stop protein standards along with samples from the same row at the same time.

Table 1: Dilution of protein standards

| | | 100ul | 100ul | 100ul | 100u | u 🔨 | |
|----------------|--------------|------------------|-----------|------------------|------------------|---------------|------------------|
| | | \sim | | | | $\gg \square$ | |
| | Added to 1st | | | | | | |
| <u>Protein</u> | <u>Strip</u> | <u>1st Strip</u> | 2nd Strip | <u>3rd Strip</u> | <u>4th Strip</u> | 5th Strip | <u>6th Strip</u> |
| Mouse IL-1a | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse IL-1b | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse G-CSF | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse GM-CSF | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse MCP-1 | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse MIP-1a | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse SCF | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse Rantes | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| | | | 1:2 | 1:4 | 1:8 | 1:16 | Blank |

Table 2: Concentrations of Protein Standard Dilutions

| <u>Protein</u> | | <u>1:2</u> | <u>1:4</u> | <u>1:8</u> | <u>1:16</u> | <u>Blank</u> |
|----------------|--------|------------|------------|------------|-------------|--------------|
| Mouse IL-1a | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse IL-1b | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse G-CSF | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse GM-CSF | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse MCP-1 | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse MIP-1a | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse SCF | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse Rantes | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |