

Mouse Obesity ELISA Strip II Protein Standards

Catalog Number EA-1422

(For Research Use Only)

| <u>Tube</u> | <u>Protein</u> | Stock Conc. | <u>Volume</u> |
|-------------|----------------|-------------|---------------|
| #1 | Mouse Leptin | 400ng/ml | 10ul |
| #2 | Mouse TNFα | 400ng/ml | 10ul |
| #3 | Mouse IGF-1 | 400ng/ml | 10ul |
| #4 | Mouse IL-6 | 400ng/ml | 10ul |
| #5 | Mouse Insulin | 400ng/ml | 10ul |
| #6 | Mouse IL-1α | 400ng/ml | 10ul |
| #7 | Mouse IL-1β | 400ng/ml | 10ul |
| #8 | Mouse Resistin | 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 of the 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. Always stop protein standards along with samples from the same row at the same time.

Table 1: Dilution of protein standards

100ul 100ul 100ul 100ul

100ul

| | Added to 1st | | | | | | |
|----------------|--------------|-----------|-----------|-----------|-----------|-----------|-----------|
| <u>Protein</u> | <u>Strip</u> | 1st Strip | 2nd Strip | 3rd Strip | 4th Strip | 5th Strip | 6th Strip |
| Mouse Leptin | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse TNFα | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse IGF-1 | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse IL-6 | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse Insulin | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse IL-1α | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse IL-1β | 4ul | 200ul | 100ul | 100ul | 100ul | 100ul | 100ul |
| Mouse Resistin | 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 Leptin | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse TNFα | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse IGF-1 | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse IL-6 | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse Insulin | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse IL-1α | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse IL-1β | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |
| Mouse Resistin | 8ng/ml | 4ng/ml | 2ng/ml | 1ng/ml | 0.5ng/ml | Blank |