

Mouse IL-10 ELISA development kit

Product Code: 3431-1A-20

CONTENTS, development kit for 20 plates:

Vial 1 (yellow top)

Monoclonal antibody 2A5 (500 μ l)

Concentration: 1 mg/ml

Vial 2 (blue top)

Biotinylated monoclonal antibody 16E3 (250 μ l)

Concentration: 1 mg/ml

Vial 3 (white top)

Streptavidin-Alkaline Phosphatase (250 μ l)

Vial 4

Recombinant mouse IL-10 standard

To ensure total recovery of stated quantity, vials have been overfilled.

STORAGE:

Shipped at ambient temperature. On arrival box 1 should be stored refrigerated at 4-8°C and box 2 should be stored frozen at -20°C.

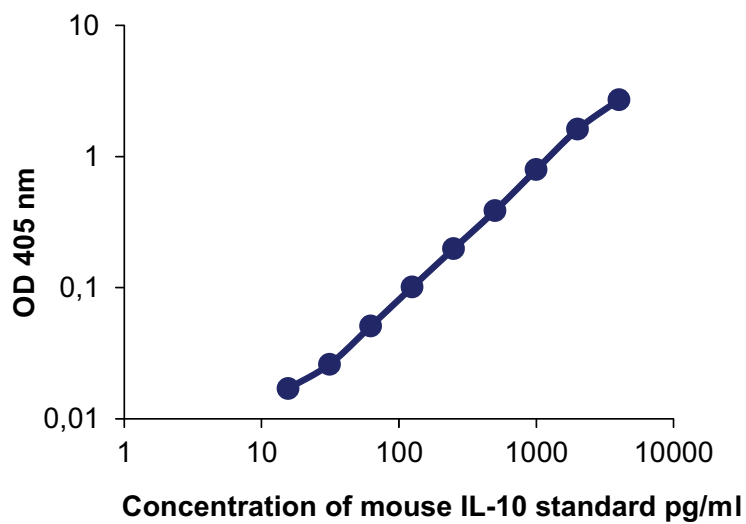
General

Intended use: For quantitative determination of native and recombinant mouse IL-10 in solution, e.g. cell culture supernatant.

Reagents: Antibodies are supplied in sterile-filtered (0.2 μm) PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG.

Standard range: 25-2500 pg/ml.

Standard calibration: No international standard exists for calibration.



Guidelines for Mouse IL-10 ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb 2A5, diluted to 2 $\mu\text{g}/\text{ml}$ in PBS, pH 7.4, by adding 100 $\mu\text{l}/\text{well}$. Incubate overnight at 4-8°C.
- Day 2**
2. Wash twice with PBS (200 $\mu\text{l}/\text{well}$).
 3. Block plate by adding 200 $\mu\text{l}/\text{well}$ of PBS with 0.05% Tween 20 containing 0.1% BSA (incubation buffer). Incubate for 1 hour at room temperature.
 4. Wash five times with PBS containing 0.05% Tween.
 5. Prepare mouse IL-10 standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA to give a concentration of 0.5 $\mu\text{g}/\text{ml}$. Leave at room temperature for 15 minutes and then vortex the tube and spin down. Use immediately or store in aliquots at -20°C for future use. We recommend the aliquots not to be refrozen after initial use. For the test, prepare dilutions of the stock using the standard range as a guideline.
 6. Add 100 $\mu\text{l}/\text{well}$ of samples or standards diluted in incubation buffer and incubate for 2 hours at room temperature. Overnight incubation at 4-8°C is recommended for optimal sensitivity.
 7. Wash as in step 4.
 8. Add 100 $\mu\text{l}/\text{well}$ of mAb 16E3-biotin at 0.1 $\mu\text{g}/\text{ml}$ in incubation buffer. Incubate for 1 hour at room temperature.
 9. Wash as in step 4.
 10. Add 100 $\mu\text{l}/\text{well}$ of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
 11. Wash as in step 4.
 12. Add 100 $\mu\text{l}/\text{well}$ of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP).
 13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

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