

# Mouse TNF- $\alpha$ ELISA development kit

Product Code: 3511-1H-20

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CONTENTS, development kit for 20 plates:

**Vial 1 (green top)**

Monoclonal antibodies MT1C8/23C9 (1000  $\mu$ l)  
Concentration: 0.5 mg/ml

**Vial 2 (yellow top)**

Biotinylated monoclonal antibody MT11B10 (250  $\mu$ l)  
Concentration: 0.5 mg/ml

**Vial 3 (white top)**

Streptavidin-Horseradish Peroxidase (250  $\mu$ l)

**Vial 4**

Recombinant mouse TNF- $\alpha$  standard (0.5  $\mu$ g)

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

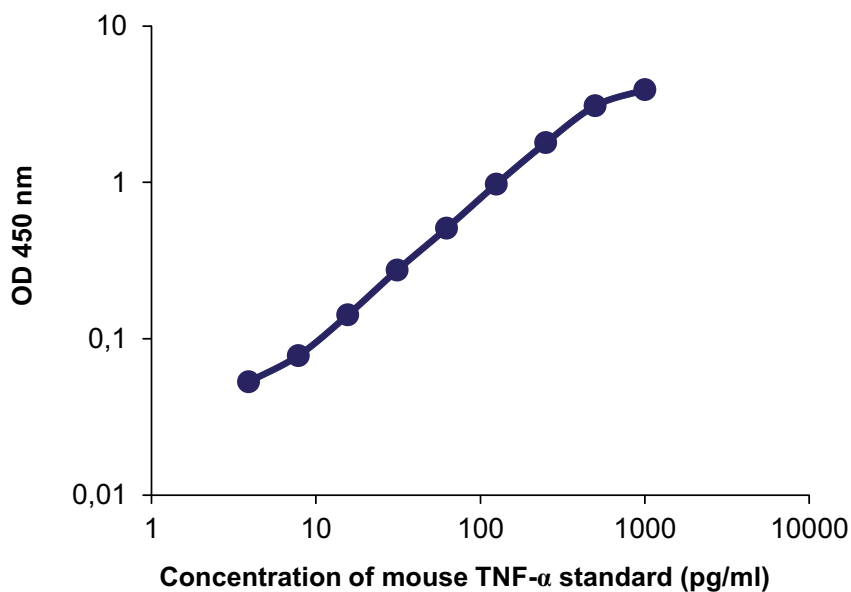
**Intended use:** For quantitative determination of native and recombinant mouse TNF- $\alpha$  in solution, e.g. cell culture supernatant.

**Reagents:** Antibodies are supplied in sterile-filtered (0.2  $\mu$ m) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.002% Kathon CG.

**Standard range:** 6-600 pg/ml

**Standard calibration:** 1 ng of supplied standard equals 209 U of 88/532 NIBSC\*-standard according to repeated calibrations. Calibration is batch-specific.

\*National Institute of Biological Standards and Control, UK.



# Guidelines for Mouse TNF- $\alpha$ ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAbs MT1C8/23C9, 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% Tween20
  5. Prepare TNF- $\alpha$  standard by reconstituting contents of vial 4 in 1 ml PBS to a concentration of 0.5  $\mu\text{g}/\text{ml}$ . Leave at room temperature for 15 minutes and then vortex the tube. The stock solution should be used immediately or stored in aliquots at -20°C for future use. We recommend the aliquots not 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.
  7. Wash as in step 4.
  8. Add 100  $\mu\text{l}/\text{well}$  of mAb MT11B10-biotin at 0.5  $\mu\text{g}/\text{ml}$  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-HRP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature. **Please note that sodium azide used in buffers will inhibit HRP activity.**
  11. Wash as in step 4.
  12. Add 100  $\mu\text{l}/\text{well}$  of appropriate substrate solution e.g. TMB, available from Mabtech product code 3652-F10.
  13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



**The products are for research use only.**

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