Mouse TNF-α ELISA development kit
Product Code: 3511-1A-20

CONTENTS, development kit for 20 plates:

**Vial 1 (green top)**
Monoclonal antibodies MT1C8/23C9 (1000 μl)
Concentration: 0.5 mg/ml

**Vial 2 (yellow top)**
Biotinylated monoclonal antibody MT11B10 (250 μl)
Concentration: 0.5 mg/ml

**Vial 3 (white top)**
Streptavidin-Alkaline Phosphatase (250 μl)

**Vial 4**
Recombinant mouse TNF-α standard (0.5 μg)

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.
**Intended use:** For quantitative determination of native and recombinant mouse TNF-α 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:** 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-α ELISA

Day 1 1. Coat a high protein binding ELISA plate with mAbs MT1C8/23C9, diluted to 2 μg/ml in PBS, pH 7.4, by adding 100 μl/well. Incubate overnight at 4-8°C.

Day 2 2. Wash twice with PBS (200 μl/well).

3. Block plate by adding 200 μl/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-α standard by reconstituting contents of vial 4 in 1 ml PBS to a concentration of 0.5 μg/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 μl/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 μl/well of mAb MT11B10-biotin at 0.5 μg/ml in incubation buffer. Incubate for 1 hour at room temperature.


10. Add 100 μl/well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.


12. Add 100 μl/well of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP), available from Mabtech product code 3652-P10.

13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.
NOTE: for research use only.

MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages therefrom.

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the above standards.

For more information about Mabtech services and our products, visit

www.mabtech.com