

# Human TNF- $\alpha$ ELISA development kit

Product Code: 3510-1H-6

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CONTENTS, development kit for 6 plates:

**Vial 1 (red top)**

Monoclonal antibody TNF3/4, a combination of two different antibodies (300  $\mu$ l)

Concentration: 0.5 mg/ml

**Vial 2 (blue top)**

Biotinylated monoclonal antibody TNF5 (150  $\mu$ l)

Concentration: 0.5 mg/ml

**Vial 3 (white top)**

Streptavidin-Horseradish Peroxidase (80  $\mu$ l)

**Vial 4**

Recombinant human TNF- $\alpha$  standard (1  $\mu$ 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:** This assay is based on the use of a combination of two coating antibodies, TNF3 and TNF4. For quantitative determination of native and recombinant human TNF- $\alpha$  in solution, e.g. cell culture supernatant.

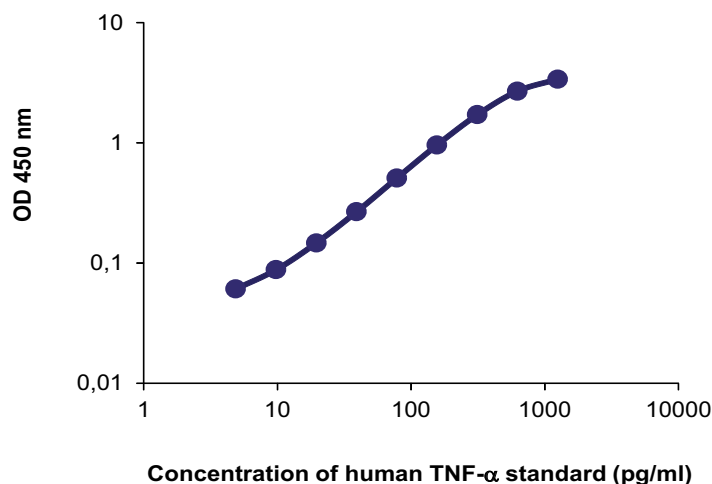
**Serum/plasma samples:** Please note that cytokine determinations in serum/plasma require the use of ELISA diluent (product code: 3652-D2) for dilution of samples, standard and detection antibody. The diluent prevents false positive read-outs which may be caused by interference of heterophilic antibodies found in plasma and serum. The ELISA diluent has been validated using serum/plasma from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions has not been assessed. Please contact Mabtech for further information.

**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:** 5-1000 pg/ml

**Standard calibration:** 1 ng of supplied standard equals 46 U of 87/650 NIBSC\*-standard according to repeated calibrations. Calibration is batch-specific.

\*National Institute of Biological Standards and Control, UK



# Guidelines for Human TNF- $\alpha$ ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb TNF3/4, 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. Incubate for 1 hour at room temperature.
  4. Wash five times with PBS containing 0.05% Tween.
  5. Prepare h TNF- $\alpha$  standard by reconstituting contents of vial 4 in 1 ml PBS to a concentration of 1  $\mu\text{g}/\text{ml}$ . 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 PBS with 0.05% Tween 20 containing **1% BSA\*** or ELISA diluent for serum/plasma samples and incubate for 2 hours at room temperature.
  7. Wash as in step 4.
  8. Add 100  $\mu\text{l}/\text{well}$  of mAb TNF5-biotin at 1  $\mu\text{g}/\text{ml}$  in PBS with 0.05% Tween 20 containing **1% BSA\*** or ELISA diluent for serum/plasma samples. 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 PBS with 0.05% Tween 20 containing 0.1% BSA. 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.

**\* High protein content is required for optimal results.**

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