

ELISA Flex: Human TNF- α (HRP)

3512-1H-6 | 3512-1H-20

ELISA Flex kit for quantitative determination of native and recombinant human TNF- α in solution, e.g. cell supernatant and serum/plasma.

The kit includes		3512-1H-6 for 6 plates	3512-1H-20 for 20 plates
Capture mAb:	MT25C5 (0.5 mg/ml)	300 μ l	1000 μ l
Detection mAb:	MT20D9, biotinylated (0.5 mg/ml)	150 μ l	500 μ l
Streptavidin-HRP		80 μ l	250 μ l
Recombinant human TNF- α ELISA standard		1 vial	1 vial
Standard reconstitution buffer A5		1 ml	1 ml

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

Shipping and storage

Shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard which should be stored at -20 °C. Antibodies are supplied in sterile-filtered PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 0.002% Kathon CG. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use.

General and Preparations

Specificity

The kit contains a matched pair of monoclonal antibodies (mAbs) specific for native and recombinant human TNF- α .

Standard range

4-400 pg/ml

Calibration

The ELISA standard has been calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. One ng of supplied standard equals 55 U of 87/650 NIBSC-standard. Please note that the calibration is batch specific.

Analysis of serum and plasma samples

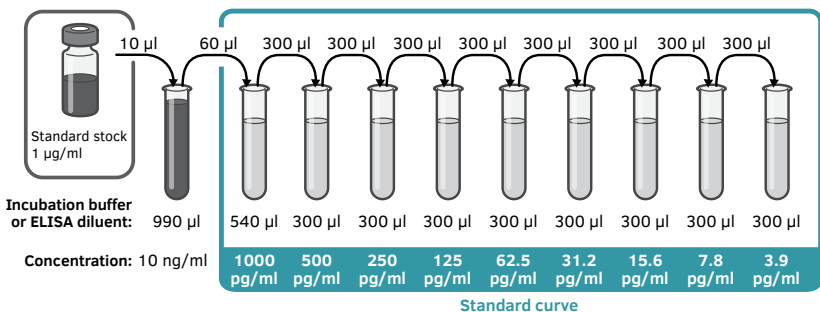
Analysis of serum/plasma requires the use of ELISA diluent (product code: 3652-D2). The ELISA diluent blocks heterophilic antibodies, commonly found in serum/plasma, from cross-linking the assay antibodies, thereby preventing false positive read-outs. The ELISA diluent should be used for dilution of standard, samples, and detection antibody.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 1 $\mu\text{g/ml}$ by adding 1 ml of the standard reconstitution buffer. Allow the standard to dissolve for 5 minutes and mix thoroughly. The standard should be kept in aliquots at -20°C . Avoid repeated freeze-thaw cycles.

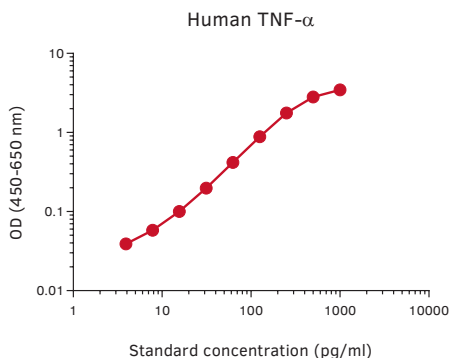
Preparation of standard curve

Prepare within 30 minutes of use. Volumes are sufficient for duplicates.



Protocol

- Day 1** **1.** Add 100 µl/well of capture mAb MT25C5 diluted to 2 µg/ml in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8 °C.
- Day 2** **2.** Empty the plate and add 200 µl/well of PBS with 0.05% Tween 20 and 0.1% BSA (incubation buffer) to block the plate. Incubate for 1 hour at room temperature.
- 3.** Wash the plate 5 times with PBS containing 0.05% Tween 20 (300 µl/well).
- 4.** Add 100 µl/well of samples or standards diluted in incubation buffer or ELISA diluent. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
- 5.** Wash as above.
- 6.** Add 100 µl/well of detection mAb MT20D9-biotin diluted to 1 µg/ml in incubation buffer or ELISA diluent. Incubate for 1 hour at room temperature.
- 7.** Wash as above.
- 8.** Add 100 µl/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.
- 9.** Wash as above.
- 10.** Add 100 µl/well of TMB substrate (product code: 3652-F10) and incubate for 15 minutes.
- 11.** Add 100 µl/well of 0.2 M H₂SO₄ to stop the reaction.
- 12.** Measure the optical density in an ELISA reader at 450 nm within 15 min. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.



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



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