

# Mouse TNF $\alpha$ ELISA-SET

Ref.: mTNF $\alpha$ -EIA-20

MabTag's ELISA for mouse TNF $\alpha$  (Tumor Necrosis Factor  $\alpha$ ) contains appropriate reagents sufficient for processing of 20 microplates (20 x 96 wells; 100  $\mu$ l/well)

For research only. Not for use in diagnostic or therapeutic procedures.

This ELISA System was evaluated with the NIBSC/WHO international reference standard 88/532

**Specificity:** mouse TNF $\alpha$  (Tumor Necrosis Factor  $\alpha$ )

**Typical standard curve range:** 47 – 3000 pg/ml

**Detection limit:** 46 pg/ml

**Samples:** Culture supernatants, serum, plasma and other body fluids.

For serum and plasma a dilution of  $\geq$  1:10 is recommended.



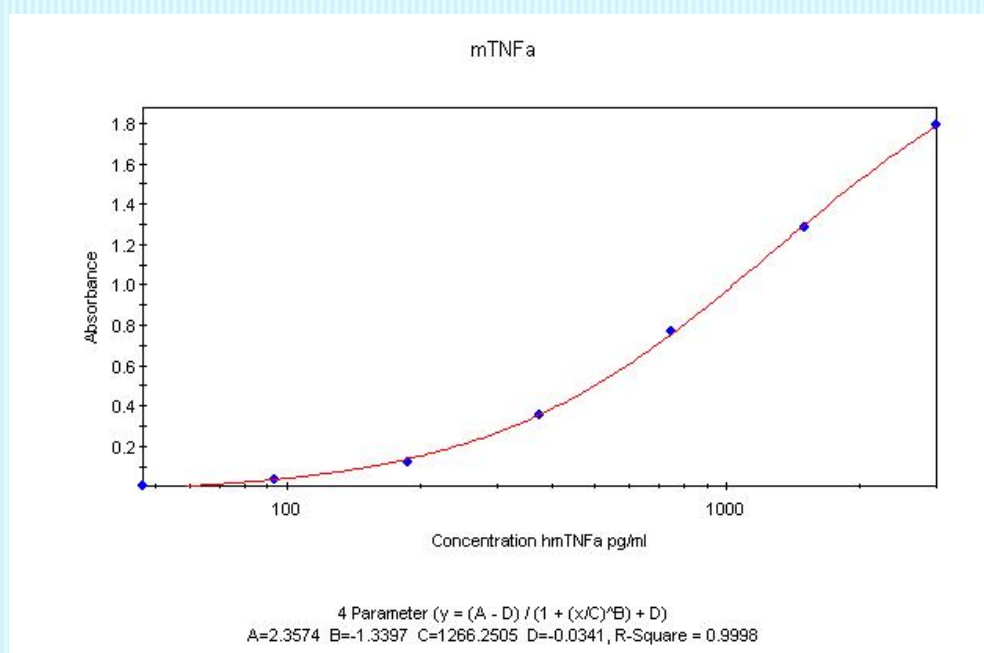
**MabTag**  
GmbH

Content	Working dilution	Storage
4 x vial 500 $\mu$ l liquid anti-mTNF $\alpha$ Capture-Antibody (red cap)	1:100	-20°C
4 x vial 500 $\mu$ l liquid anti-mTNF $\alpha$ Detection-Antibody (yellow cap)	1:100	-20°C
4 x vial 50 ng lyophilized rmTNF $\alpha$ Standard (white cap)	customer specific	-20°C
4 x vial 50 $\mu$ l Poly-HRP-Streptavidin (blue or green cap)	1:1000	-20°C

## Additional material required: General ELISA Reagent Pack (GenEIA-Pack-5/20) or

96well-Microplate
Coating-Buffer (e.g. PBS)
Blocking-Buffer / Reagent-Diluent (e.g. PBS + 2% BSA + 0.05% Tween20)
Wash-Buffer (e.g. PBS + 0.05% Tween20)
TMB-Substrate
Stop-solution (e.g. 2 M H <sub>2</sub> SO <sub>4</sub> )

## Typical standard curve



**!Spin down all vials before use!**

Step	Incubation	Procedure
<b>Coating Capture-antibody</b>	≥ OVERNIGHT at room temperature	Dilute <b>capture-antibody</b> 1:100 in COATING-BUFFER (100 µl capture-antibody in 10 ml COATING-BUFFER). Subsequently transfer 100 µl of this working-solution to each well and incubate.
Remove capture-antibody completely by inverting the microplate and blotting it <b>vigorously</b> against clean paper towels.		
<b>Blocking</b>	≥ 1 Hour at room temperature	Add 300 µl BLOCKING-BUFFER to each well and incubate.
Remove BLOCKING-BUFFER completely by inverting the microplate and blotting it <b>vigorously</b> against clean paper towels.		
<b>Standard &amp; Sample</b>	≥ 2 Hours at room temperature	Dilute <b>standard</b> & samples in REAGENT-DILUENT and transfer 100 µl in the respective wells in duplicates. Standard: Make serial dilutions in REAGENT-DILUENT and begin with a high standard and end with blanks. The standard vial of this set contains <b>50 ng lyophilized standard</b> . Reconstitute this in exactly 1 ml REAGENT-DILUENT (stock solution = 50 ng/ml) and choose a sufficient high standard working solution for your assay (e.g. prepare a 1:100 dilution for a standard curve beginning with 500 µg/ml).
Wash 5x <b>vigorously</b> with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it <b>vigorously</b> against clean paper towels.		
<b>Detection- antibody</b>	≥ 2 Hours at room temperature	Dilute <b>detection-antibody</b> 1:100 in REAGENT-DILUENT (100 µl detection-antibody in 10 ml REAGENT-DILUENT). Subsequently transfer 100 µl of this working-solution to each well and incubate.
Wash 5x <b>vigorously</b> with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it <b>vigorously</b> against clean paper towels.		
<b>Poly-HRP- Streptavidin</b>	<b>20-30 Min</b> at room temperature	Dilute <b>Poly-HRP-Streptavidin</b> 1:1000 in REAGENT-DILUENT (10 µl in 10 ml REAGENT-DILUENT). Subsequently transfer 100 µl of this working-solution to each well and incubate.
Wash 5x <b>vigorously</b> with WASHING-BUFFER and remove resting buffer completely by inverting the microplate and blotting it <b>vigorously</b> against clean paper towels.		
<b>Substrate solution</b>	Up to 60 Min* at room temperature <b>in the dark</b>	Optionally warm the solution to room temperature before use. Add 100 µl of the SUBSTRATE-SOLUTION to each well and incubate. Control the development of the colour reaction continuously and stop at an appropriate time point.
<b>Stop solution</b>	-	When the enzymatic colour reaction is sufficiently proceeded stop the reaction by adding of 50 µl stop solution. Read the microplate at the substrate-depending wavelength. (e.g. <b>450 nm</b> for TMB substrate) (if wavelength correction is available, set to 540 nm, 570 nm or 630 nm as reference)

\*The speed of enzymatic colour development is influenced by many customer-specific factors. Therefore the incubation time is variable und specific for each test system.

**Note:**

All incubation steps except Poly-HRP-Streptavidin and TMB substrate could be optionally carried out over-night. Do not use sodium azide-containing solutions, nor add sodium azide to the supplied reagents. Sodium azide inactivates the peroxidase.

**Storage:**

Specific storage conditions in the table above.

Reconstituted reagents should be stored at -20°C. Please prevent repeated freeze- thaw cycles. Stable for up to 6 months after opening when stored at -20° C. The performance of the unopened reagents is guaranteed until one year after point of delivery.

**Precautions for use:**

!The stop solution is an acid solution. TMB-Solution A contain H<sub>2</sub>O<sub>2</sub> and tetramethylbenzidine (TMB). All Buffers and liquid antibody solutions contain 0.045% (v/v) Proclin®950 as preservative. All these compounds are harmful and cause respiratory, skin and eye irritation. Do not swallow any components of the set/kit (R22). Wear face, eye and hand clothing protection when using this material (S36). Keep out of reach of children (S2). Keep away from food, drink and animal feeding stuff (S13). !These reagents are offered for research purposes only! For *in vitro* use only. MabTag will not be held responsible for patent infringement or other violations that may occur with the use of our products.

ProClin is a registered trade mark of Rohm and Haas Company.