Porcine TNF-a ELISA development kit

Product Code: 3135-1H-20

CONTENTS, development kit for 20 plates:

Vial 1 (red top) Monoclonal antibody MT1P14 (1000 µl) Concentration: 0.5 mg/ml

Vial 2 (yellow top) Biotinylated monoclonal antibody MT859 (250 µl) Concentration: 0.5 mg/ml

Vial 3 (white top) Streptavidin-Horseradish Peroxidase (250 µl)

Vial 4 Recombinant porcine TNF-α standard

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.

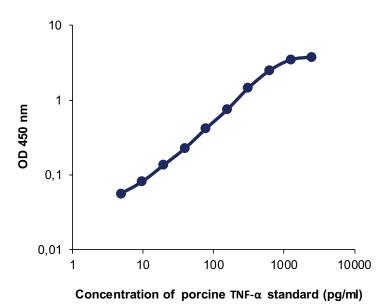
General

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

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

Standard range: 10-1000 pg/ml

Standard calibration: No international standard exists for calibration.



Guidelines for Porcine TNF-a ELISA

- **Day 1** 1. Coat a high protein binding ELISA plate with mAb MT1P14, 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 ($200 \mu l/well$).
 - 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 porcine TNF-α standard by reconstituting contents of vial 4 in 1 ml PBS to give a concentration of 2.5 μg/ml. Leave at room temperature for 15 minutes, then vortex the tube and spin down. Use immediately or store in aliquots at -20°C for future use. We recommend the aliquots not to 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 μ /well of mAb MT859-biotin at 0.5 μ g/ml in incubation buffer. Incubate for 1 hour at room temperature.
 - 9. Wash as in step 4.
 - 10. 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.**
 - 11. Wash as in step 4.
 - 12. Add 100 μl/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.



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