

## ELISA Flex: Porcine TNF- $\alpha$ (HRP)

3135-1H-6 | 3135-1H-20

ELISA Flex kit for quantitative determination of native and recombinant porcine TNF- $\alpha$  in solution, e.g. cell supernatant.

The kit includes		3135-1H-6 for 6 plates	3135-1H-20 for 20 plates
Capture mAb:	MT1P14 (0.5 mg/ml)	300 $\mu$ l	1000 $\mu$ l
Detection mAb:	MT859, biotin (0.5 mg/ml)	80 $\mu$ l	250 $\mu$ l
Streptavidin-HRP		80 $\mu$ l	250 $\mu$ l
Recombinant porcine TNF- $\alpha$ 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 porcine TNF- $\alpha$ .

## Standard range

10-1000 pg/ml

## Calibration

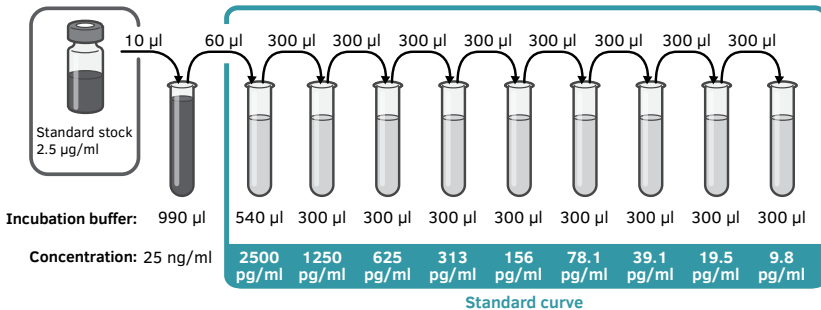
No international standard exists for calibration.

## Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 2.5  $\mu\text{g/ml}$  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  $-20^\circ\text{C}$ . Avoid repeated freeze-thaw cycles.

## Preparation of standard curve

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



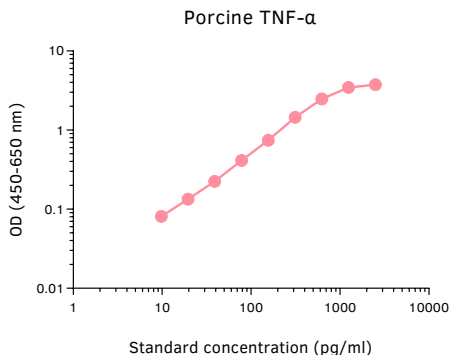
# Protocol

## Day 1

1. Add 100  $\mu\text{l}$ /well of capture mAb MT1P14 diluted to 2  $\mu\text{g}/\text{ml}$  in PBS, pH 7.4. Use high protein binding ELISA plates. Incubate overnight at 4-8  $^{\circ}\text{C}$ .

## Day 2

2. Empty the plate and add 200  $\mu\text{l}$ /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  $\mu\text{l}$ /well).
4. Add 100  $\mu\text{l}$ /well of samples or standards diluted in incubation buffer. Include assay background control, i.e. wells without standard. Incubate for 2 hours at room temperature.
5. Wash as above.
6. Add 100  $\mu\text{l}$ /well of detection mAb MT859-biotin diluted to 0.5  $\mu\text{g}/\text{ml}$  in incubation buffer. Incubate for 1 hour at room temperature.
7. Wash as above.
8. Add 100  $\mu\text{l}$ /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  $\mu\text{l}$ /well of TMB substrate (product code: 3652-F10) and incubate at room temperature, protected from direct light for 15 minutes.
11. Add 100  $\mu\text{l}$ /well of 0.2 M  $\text{H}_2\text{SO}_4$  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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