## Datasheet & Protocol

# MABTECH

# ELISA Flex: Mouse IL-1α (ALP)

3417-1A-6 | 3417-1A-20

ELISA Flex kit for quantitative determination of native and recombinant mouse IL-1 $\alpha$  in solution, e.g. cell supernatant and serum/plasma.

The kit includes	<b>3417-1A-6</b> for 6 plates	<b>3417-1A-20</b> for 20 plates
Capture mAb: MT946 (0.5 mg/ml)	300 μl	1000 μl
Detection mAb: MT951, biotin (0.5 mg/ml)	80 µl	250 µl
Streptavidin-ALP	80 µl	250 µl
Recombinant mouse IL-1α 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-ALP is supplied in 0.1 M Tris buffer 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 mouse IL-1 $\alpha$ . The assay is specific for mouse IL-1 $\alpha$  and shows no cross-reactivity with recombinant mouse IL-1 $\beta$ .

Standard range 2-250 pg/ml

Calibration

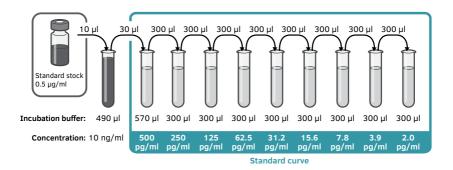
No international standard exists for calibration.

#### **Reconstitution of ELISA standard**

Reconstitute the ELISA standard to a stock solution of 0.5  $\mu$ 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.



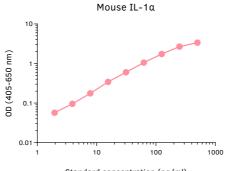
### Protocol

Day 1

1. Add 100  $\mu$ /well of capture mAb MT946 diluted to 2  $\mu$ g/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  $\mu$ /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  $\mu$ l/well).
- **4.** Add 100 μl/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  $\mu$ /well of detection mAb MT951-biotin diluted to 0.5  $\mu$ g/ml in incubation buffer. Incubate for 1 hour at room temperature.
- 7. Wash as above.
- **8.** Add 100 μl/well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
- 9. Wash as above.
- **10.** Add 100 μl/well of pNPP substrate (product code: 3652-P10) and incubate at room temperature protected from direct light for approximately 60 minutes.
- **11.** Measure the optical density in an ELISA reader at 405 nm. Preferably use a reader capable of subtracting a reference wavelength of between 570 and 650 nm. Representative standard curve shown below.



Standard concentration (pg/ml)



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



#### The products are for research use only.

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