MABTECH

ELISA Flex: Mouse IL-4 (ALP)

3311-1A-6 | 3311-1A-20

ELISA Flex kit for quantitative determination of native mouse IL-4 in solution, e.g. cell supernatant and serum/plasma samples.

The kit includes		3311-1A-6 for 6 plates	3311-1A-20 for 20 plates
Capture mAb:	11B11 (1 mg/ml)	150 µl	500 µl
Detection mAb:	BVD6-24G2, biotin (1 mg/ml)	80 µl	250 µl
Streptavidin-ALP		80 µl	250 µl
Recombinant mouse IL-4 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-4.

Standard range

4-400 pg/ml

Calibration

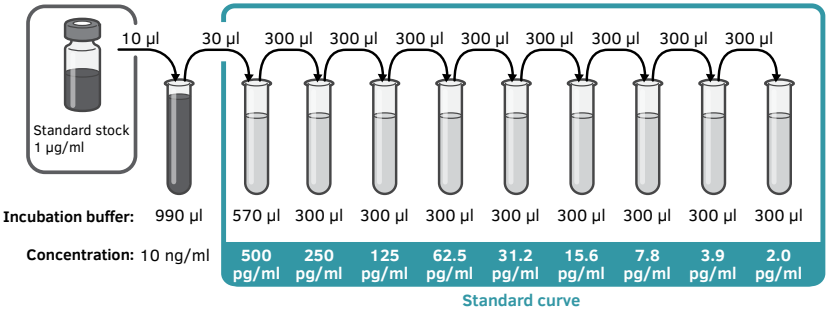
The ELISA standard has been calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. One ng of supplied standard equals 25 U of 91/656 NIBSC-standard. Please note that the calibration is batch specific.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 1 µg/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.



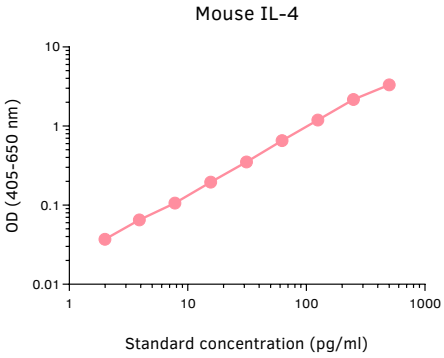
Protocol

Day 1

1. Dilute capture mAb 11B11 to 2 µg/ml in PBS, pH 7.4, and filter the solution through a 0.2 µm filter. Add 100 µl/well. 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. 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 BVD6-24G2-biotin diluted to 0.1 µg/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.



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