MABTECH

ELISA Flex: Human IgM (ALP)

3880-1AD-6 I

ELISA Flex kit for quantitative determination of native human IgM in solution, e.g. serum/plasma samples or cell supernatant.

The kit includes		3880-1AD-6 for 6 plates
Capture mAbs:	MT11/12 (0.5 mg/ml)	300 µl
Detection mAb:	MT22, ALP	80 µl
Human IgM ELISA standard		1 vial
Standard reconstitution buffer A5		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%). The detection antibody is supplied in 0.1 M Tris-buffer with 1% BSA and 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 human IgM. The mAbs cross-react with IgM from non-human primates (NHP). Please visit www.mabtech.com for reactivity on NHP species.

Standard range

0.2-200 ng/ml

Calibration

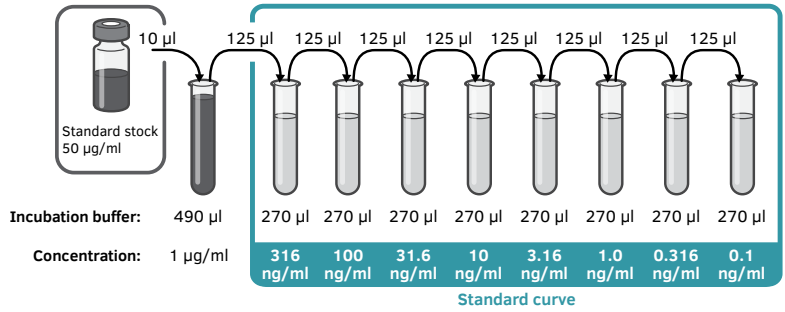
The ELISA standard has been calibrated against an international standard from the National Institute of Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire EN6 3QG, UK. One µg of supplied standard equals 233 mU NIBSC-standard. Please note that the calibration is batch specific.

Reconstitution of ELISA standard

Reconstitute the ELISA standard to a stock solution of 50 µg/ml by adding 0.5 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.



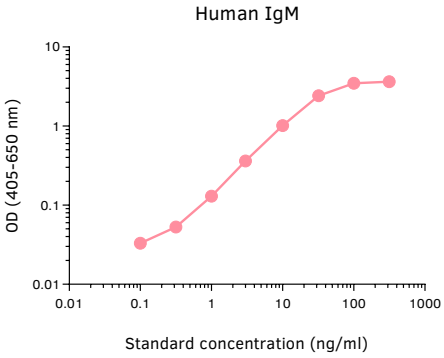
Protocol

Day 1

1. Add 100 µl/well of capture mAbs MT11/12 diluted to 2 µg/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 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 MT22-ALP diluted 1:1000 in incubation buffer. Incubate for
1 hour at room temperature.
7. Wash as above.
8. Add 100 µl/well of pNPP substrate (product code: 3652-P10) and incubate the plate for approximately 60 minutes.
9. 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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The products are for research use only.
MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages there from.

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