Human IgM ELISA development kit

Product Code: 3840-1AD-6

CONTENTS:

Vial 1 (red top)
Monoclonal anti-IgM antibody (300 µl)
Concentration: 0.5 mg/ml

Vial 2 (yellow top)
ALP-conjugated anti-IgM antibody (80 µl)

Vial 3
Lyophilised human IgM 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

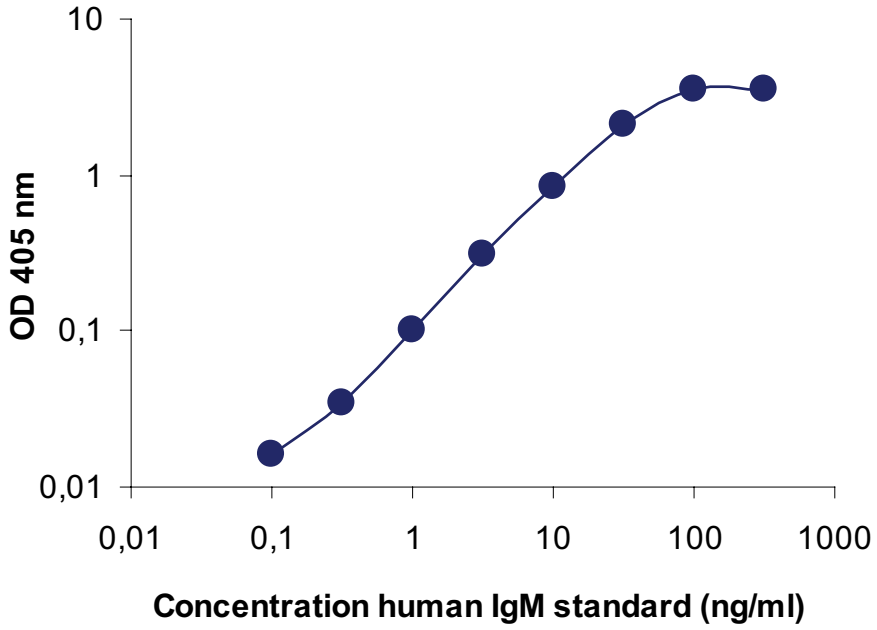
Intended use: For quantitative determination of native human IgM in serum and plasma.

Reagents: Anti-IgM monoclonal antibody is supplied in sterile-filtered (0.2 µm) PBS with sodium azide (0.02%). ALP-conjugated anti-IgM antibody is supplied in 0.1 M Tris-buffer with 0.002% Kathon CG.

Recommended standard dilution: 0.1-500 ng/ml

Standard calibration: 1 µg of supplied standard equals 233 mU NIBSC* standard. Calibration is batch-specific.

* National Institute of Biological Standards and Control, UK.



Guidelines for Human IgM ELISA

Day 1 1. Coat a high protein binding ELISA plate with anti-IgM monoclonal antibody, 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 µl/well).

3. Block plate by adding 200 µl/well of PBS with 0.05% Tween 20 (PBS-Tween) containing 0.1% BSA (incubation buffer*). Incubate for 1 hour at room temperature.

4. Wash five times with PBS-Tween.

5. Prepare human IgM standard by reconstituting contents of vial 3 in 500 µl PBS to make up a stock solution of 50 µg/ml. The stock solution should be used immediately or stored in aliquots at -20°C for future use. We recommend the aliquots not 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. It is recommended to make serial dilutions of samples to obtain results within the standard range. Incubate for 2 hours at room temperature.

7. Wash as in step 4.

8. Add 100 µl/well of anti-IgM-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.

9. Wash as in step 4.

10. Add 100 µl/well of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP).

11. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

* The same buffer is used for blocking and for dilution.

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