Human Apolipoprotein E ELISA development kit

Product Code: 3712-1H-6

CONTENTS, development kit for 6 plates:

Vial 1 (red top)
Monoclonal antibody E276 (300 µl)
Concentration: 0.5 mg/ml

Vial 2 (yellow top)
Biotinylated monoclonal antibody E887 (150 µl)
Concentration: 0.5 mg/ml

Vial 3 (white top)
Streptavidin-Horseradish peroxidase (80 µl)

Vial 4
Lyophilised recombinant apoE3 standard (5 µg)

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

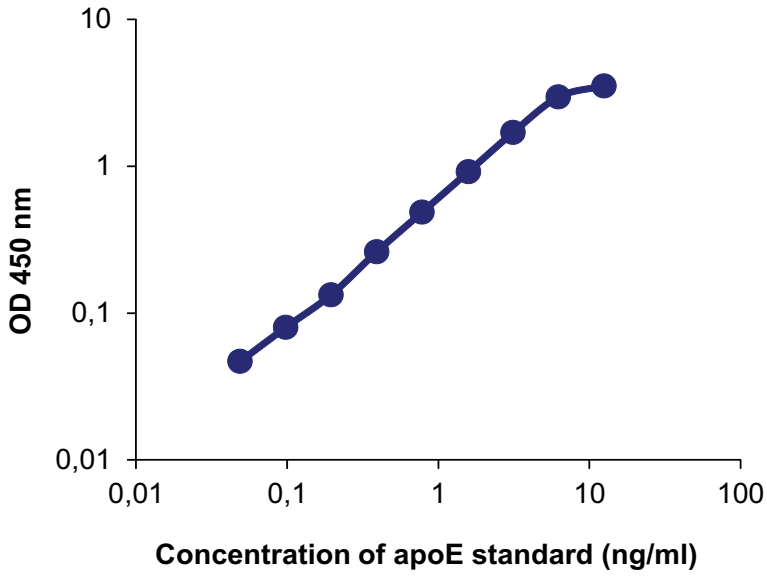
Intended use: For quantitative determination of human Apolipoprotein E (apoE) in serum/plasma samples and cell culture supernatants. The mAbs detect the three apoE isoforms apoE2, apoE3 and apoE4.
Please note that wash-, block- and incubation buffers should contain detergent. Tween 20, Triton X-100 or NP40 can be used at a concentration of 0.05-0.5%. In block and incubation buffers it is recommended to use 0.1% BSA.

Serum/plasma samples: When analyzing human serum/plasma samples it is recommended to use Apo ELISA buffer (product code: 3652-M2) for dilution of samples, standard and detection antibody. The buffer prevents false positive read-outs which may be caused by interference of heterophilic antibodies commonly found in human plasma and serum. The Apo ELISA buffer has been validated using serum/plasma from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions has not been assessed. Serum and plasma samples containing EDTA, citrate or heparin may be used. However, heparin containing samples will give higher apoE values due to displacement of proteoglycan bound apoE. Triton X-treatment of samples, necessary for apoB analysis, will not interfere with apoE analysis. It is recommended to dilute serum/plasma samples 10,000x to 20,000x, see dilution guidelines at https://www.mabtech.com/knowledge-center/apodilution. Avoid repeated freezing-thawing cycles.

Reagents: Antibodies are supplied in sterile-filtered (0.2 µm) PBS with sodium azide (0.02%). Streptavidin-HRP is supplied in PBS with 1% BSA and 0.002% Kathon CG.

Standard range: 0.1-10 ng/ml

Standard calibration: No international standard exists for calibration.



Guidelines for Human Apolipoprotein E ELISA

Day 1

1. Coat a high protein binding ELISA plate with mAb E276, 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 containing 0.1% BSA (incubation buffer). Incubate for 1 hour at room temperature.
4. Wash five times with PBS containing 0.05% Tween.
5. Prepare apoE standard by reconstituting contents of vial 4 in 1 ml PBS with 0.5 mM DTT and 0.1% BSA, do not stir and leave at room temperature for 20 minutes. This gives a stock solution of 5 µg/ml which should be used immediately or stored in aliquots at -20°C for future use. We recommend the aliquots not to 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 or Apo ELISA buffer for serum/plasma samples and incubate for 1 to 2 hours at room temperature. Dilution recommendations for serum/plasma samples can be found at https://www.mabtech.com/knowledge-center/apodilution.
7. Wash as in step 4.
8. Add 100 µl/well of mAb E887-biotin at 1 µg/ml in incubation buffer or Apo ELISA buffer for serum/plasma samples. Incubate for 1 hour at room temperature
9. Wash as in step 4.
10. Add 100 µl/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.**
11. Wash as in step 4.
12. Add 100 µl/well of appropriate substrate solution e.g. TMB, available from Mabtech product code 3652-F10.
13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.

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CERTIFIED ISO 9001 Quality management systems

CERTIFIED ISO 13485 Medical devices Quality management systems

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