ELISA for Human Apolipoprotein E

Product Code: 3712-1A-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-Alkaline Phosphatase (80 µl)

Vial 4
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

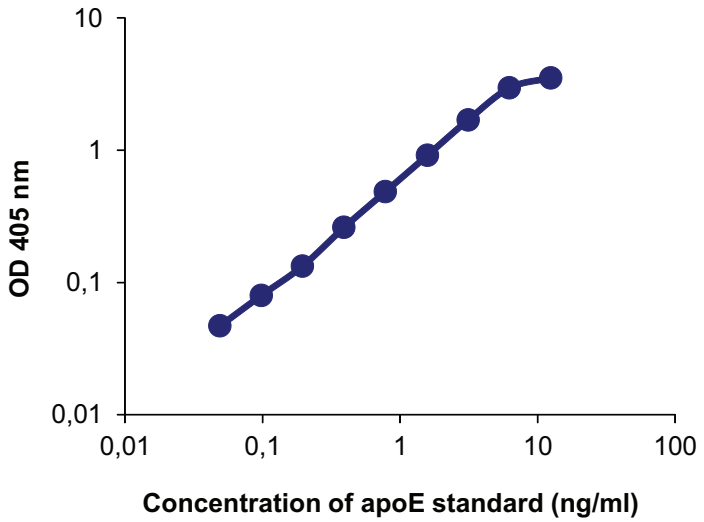
Intended use: For quantitative determination of native and recombinant human Apolipoprotein E in serum/plasma samples and cell culture supernatants. The mAb pair detects the three apoE isoforms apoE2, apoE3 and apoE4. Serum and plasma samples containing EDTA, citrate or heparin may be used. However, heparin containing samples will give higher apoE values due to displacement of proteoglycan bound apoE.

Serum/plasma samples: The mAbs will recognize apoE in human serum/plasma only in the presence of non-ionic detergents at a concentration of 0.01-0.2%. Avoid vortex in the presence of detergent. We recommend the use of Assay buffer (product code: 3652-J2) for dilution of samples, standard and detection antibody. The buffer also prevents false positive read-outs which may be caused by interference of heterophilic antibodies commonly found in human plasma and serum. The Assay 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. Please contact Mabtech for further information.

Reagents: Antibodies are supplied in sterile-filtered (0.2 µm) PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.15% 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. It is important to wait 20 minutes before resuspending the liquid. This gives a stock solution of 5 µg/ml which should be used immediately or stored in aliquots at -20°C for future use. The recommended standard dilutions range from 0.1-10 ng/ml.
6. Add 100 µl/well of samples or standards diluted in incubation buffer or Assay buffer for serum/plasma samples and incubate for 1 to 2 hours at room temperature.
7. Wash as in step 4.
8. Add 100 µl/well of mAb E887-biotin at 1 µg/ml in incubation buffer or Assay 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-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
11. Wash as in step 4.
12. Add 100 µl/well of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP).
13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

NOTE; 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 therefrom.

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