CONTENTS, development kit for 6 plates:

Vial 1 (red top)
Monoclonal antibody E981 (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
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 monkey Apolipoprotein E (apoE) in serum/plasma samples and cell culture supernatants. 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 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 plasma and serum. The Apo ELISA buffer has been validated using serum/plasma from normal healthy human blood donors. 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. Please 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-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG.

**Standard range:** 0.1-10 ng/ml

**Standard calibration:** No international standard exists for calibration.
Guidelines for Monkey Apolipoprotein E ELISA

Day 1
1. Coat a high protein binding ELISA plate with mAb E981, 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.
10. Add 100 μl/well of Streptavidin-ALP diluted 1:1000 in incubation buffer. Incubate for 1 hour at room temperature.
12. Add 100 μl/well of appropriate substrate solution e.g. p-nitrophenyl-phosphate (pNPP), available from Mabtech product code 3652-P10.
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.

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the above standards.

For more information about Mabtech services and our products, visit

www.mabtech.com