Amplite™ Monkey Apolipoprotein E (ApoE) Kit *Optimized For ELISA Development with HRP*

Catalog number: V101035
Unit size: 96 Tests

Component	Storage	Amount
Biotinylated monoclonal antibody E887	Refrigerated (2-8 °C), Minimize light exposure	1 vial (150 uL, 0.5 mg/mL)
Lyophilised recombinant apoE3 standard	Freeze (< -15 °C), Minimize light exposure	1 vial (5 ug)
Monoclonal antibody E981	Refrigerated (2-8 °C), Minimize light exposure	1 vial (300 uL, 0.5 mg/mL)
Streptavidin-Horseradish Peroxidase	Refrigerated (2-8 °C), Minimize light exposure	1 vial (80 uL)

AT A GLANCE

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.aatbio.com/tools/serial-dilution/ . Avoid repeated freezing-thawing cycles.

Note Apo ELISA buffer is not provided in this kit. It can be purchased from MabTech (product code: 3652-M2).

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

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

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.

SAMPLE EXPERIMENTAL PROTOCOL

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.
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.aatbio.com/tools/serial-dilution/ .
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.

    Note available from AAT Bioquest, Cat# 11012
13. Measure the optical density in an ELISA reader after suitable developing time. If required stop the reaction first.

EXAMPLE DATA ANALYSIS AND FIGURES

Example data analysis and images of this product can be found on the web at: https://www.aatbio.com/products/amplite-monkey-apolipoprotein-e-apoe-kit-optimized-for-elisa-development-with-hrp

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.