Amplite™ Human Apolipoprotein A1 (ApoA1) Kit *Optimized For ELISA Development with HRP*

Catalog number: V101005
Unit size: 96 Tests

Component	Storage	Amount
Biotinylated monoclonal antibody HDL 44	Refrigerated (2-8 °C), Minimize light exposure	1 vial (80 uL, 1mg/mL)
Lyophilised purified apoA1 standard batch 9	Freeze (< -15 °C), Minimize light exposure	1 vial (4 ug)
Monoclonal Antibody HDL 110	Refrigerated (2-8 °C), Minimize light exposure	1 vial (150 uL, 1mg/mL)
Streptavidin-Horseradish Peroxidase	Refrigerated (2-8 °C), Minimize light exposure	1 vial (80 uL)

AT A GLANCE

Intended use: For quantitative determination of human Apolipoprotein A1 (apoA1) 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, but not bovine serum, as HDL 44 also binds bovine apoA1.

Serum/plasma samples: When analyzing human serum/plasma samples it is recommended to use Apo ELISA buffer for dilution of samples, standard and detection an- tibody. The buffer prevents false positive read-outs which may be caused by interference of hetero- philic antibodies commonly found in human plasma and serum. Triton X-treatment of samples, necessary for apoB analysis, will not interfere with apoA1 analysis. It is recommended to dilute se- rum/plasma samples 150,000x to 200,000x. Avoid repeated freezing-thawing cycles and do not store samples in -20°C. Samples stored in -20°C will give false high apoA1 values.

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: 0.6-40 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 apoA1 standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA, do not stir and leave at room temperature for 15 minutes followed by vortex for 3 sek. This gives a stock solution of 4 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 HDL 110, 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 apoA1 standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA, do not stir and leave at room temperature for 15 minutes followed by vortex for 3 sek. This gives a stock solution of 4 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 stand- ard range as a guideline.
6. Prepare apoA1 standard by reconstituting contents of vial 4 in 1 ml PBS with 1% BSA, do not stir and leave at room temperature for 15 minutes followed by vortex for 3 sek. This gives a stock solution of 4 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 stand- ard range as a guideline.
7. Wash as in step 4.
8. Add 100 µl/well of mAb HDL 44-biotin at 0.5 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-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.

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-human-apolipoprotein-a1-apoa1-kit-optimized-for-elisa-development-with-hrp

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