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

Catalog number: V101000
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_Alkaline Phosphatase	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 antibody. The buffer prevents false positive read-outs which may be caused by interference of heterophilic 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, see dilution guidelines at https://www.mabtech.com/ knowledge-center/apodilution. Avoid repeated freezing-thawing cycles and do not store samples in -20°C. Samples stored in -20°C will give false high apoA1 values.

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

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: 0.6-40 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 stand- ard range as a guideline.

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-alp

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. 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 HDL 44-biotin at 0.5 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-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), available from AAT Bioquest, Cat#11619
13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

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.