

Human Apolipoprotein A1 ELISA development kit

Product Code: 3710-1A-6

CONTENTS, development kit for 6 plates:

Vial 1 (green top)

Monoclonal antibody HDL 110 (150 μ l)

Concentration: 1 mg/ml

Vial 2 (yellow top)

Biotinylated monoclonal antibody HDL 44 (80 μ l)

Concentration: 1 mg/ml

Vial 3 (white top)

Streptavidin-Alkaline Phosphatase (80 μ l)

Vial 4

Lyophilised purified apoA1 standard batch 9 (4 μ g)

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 human Apolipoprotein A1 (apoA1) in serum/plasma samples and cell culture supernatants.

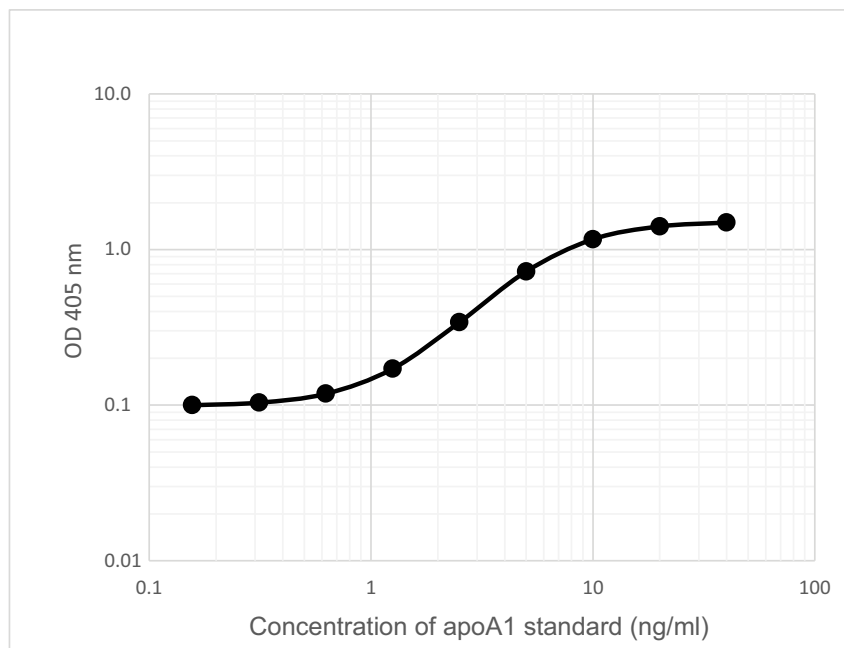
Please note that wash-, block- and incubation buffers should contain detergent. Tween 20, Triton X-100 or NP40 can be used at a concentration of 0.05-0.5%. In block and incubation buffers it is recommended to use 0.1% BSA, but not bovine serum, as HDL 44 also binds bovine apoA1.

Serum/plasma samples: When analyzing human serum/plasma samples it is recommended to use Apo ELISA buffer (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 human plasma and serum. Triton X-treatment of samples, necessary for apoB analysis, will not interfere with apoA1 analysis. It is recommended to dilute serum/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.

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

Standard calibration: The standard has been calibrated against an international standard from WHO. One ng of supplied standard equals one ng of WHO-IFCC:SP1-01 standard. Please note that the calibration is batch specific.



Guidelines for Human Apolipoprotein A1 ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb HDL 110, diluted to 2 $\mu\text{g}/\text{ml}$ in PBS, pH 7.4, by adding 100 $\mu\text{l}/\text{well}$. Incubate overnight at 4-8°C.
- Day 2**
2. Wash twice with PBS (200 $\mu\text{l}/\text{well}$).
 3. Block plate by adding 200 $\mu\text{l}/\text{well}$ 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 $\mu\text{g}/\text{ml}$ 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 $\mu\text{l}/\text{well}$ 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 $\mu\text{l}/\text{well}$ of mAb HDL 44-biotin at 0.5 $\mu\text{g}/\text{ml}$ 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 $\mu\text{l}/\text{well}$ 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 $\mu\text{l}/\text{well}$ 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.

Developed and manufactured by MABTECH AB, Sweden, whose quality management system complies with the standards ISO 9001:2015 & ISO 13485:2016.



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