Human Apolipoprotein A1 ELISA development kit
Product Code: 3710-1A-20

CONTENTS, development kit for 20 plates:

**Vial 1 (green top)**
Monoclonal antibody HDL 110 (500 μl)
Concentration: 1 mg/ml

**Vial 2 (yellow top)**
Biotinylated monoclonal antibody HDL 44 (250 μl)
Concentration: 1 mg/ml

**Vial 3 (white top)**
Streptavidin-Alkaline Phosphatase (250 μl)

**Vial 4**
Lyophilised purified apoA1 standard batch 9 (4 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.
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 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 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.
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.
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.

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

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