

Amplite™ Human Apolipoprotein B (ApoB) Kit

Optimized For ELISA Development with HRP

Catalog number: V101055
Unit size: 96 Tests

Component	Storage	Amount
Biotinylated monoclonal antibody LDL 11	Refrigerated (2-8 °C), Minimize light exposure	1 vial (80 uL, 1 mg/mL)
Monoclonal antibody LDL 20/17	Refrigerated (2-8 °C), Minimize light exposure	1 vial (150 uL, 1 mg/mL)
Purified apoB standard	Freeze (< -15 °C), Minimize light exposure	
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 B (apoB) in serum/ plasma samples or cell culture supernatants. This kit is specific for the detection of apoB100 and does not recognize apoB48. The mAbs recognize apoB100 in its form as VLDL/LDL associated protein, whereas purified delipidified apoB is poorly recognized. 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: To prevent interference by different LDL-particle sizes, serum/plasma samples should be treated with Triton X-100. Dilute samples 2x with 1% Triton X followed by vortex for 5 seconds. Triton X-treatment is not necessary for the apoB standard or for cell line produced 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. The Apo ELISA buffer has been validated using serum/plasma from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions has not been assessed. Freezing and thawing serum/plasma will reduce the recognition of apoB by these antibodies. It is recommended to dilute serum/plasma samples 5,000x to 8,000x. Please see dilution guidelines at <https://www.aatbio.com/tools/serial-dilution/>.

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: 8-800 ng/ml

SAMPLE EXPERIMENTAL PROTOCOL

- Coat a high protein binding ELISA plate with mAb LDL 20/17, diluted to 2 µg/ml in PBS, pH 7.4, by adding 100 µl/well. Incubate overnight at 4-8°C.
- Wash twice with PBS (200 µl/well).
- 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.
- Wash five times with PBS containing 0.05% Tween.
- The apoB standard is supplied as purified LDL stabilised by 50% glycerol. The concentration is 125 µg/ml. For the test, prepare dilutions of the stock using the standard range as a guideline.
- 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. Please note the special considerations

for serum/plasma samples described above. Dilution recommendations for serum/plasma samples can be found at <https://www.aatbio.com/tools/serial-dilution/>.

- Wash as in step 4.
- Add 100 µl/well of mAb LDL 11-biotin at 1 µg/ml in incubation buffer or Apo ELISA buffer for serum/plasma samples. Incubate for 1 hour at room temperature.
- Wash as in step 4.
- 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.
- Wash as in step 4.
- Add 100 µl/well of appropriate substrate solution e.g. TMB, available from AAT Bioquest, [Cat# 11012](#).
- 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-b-apob-kit-optimized-for-elisa-development-with-hrp>

DISCLAIMER

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