

# Human Apolipoprotein B ELISA development kit

Product Code: 3715-1A-6

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CONTENTS, development kit for 6 plates:

**Vial 1 (blue top)**

Monoclonal antibody LDL 20/17 (150  $\mu$ l)

Concentration: 1 mg/ml

**Vial 2 (red top)**

Biotinylated monoclonal antibody LDL 11 (80  $\mu$ l)

Concentration: 1 mg/ml

**Vial 3 (white top)**

Streptavidin-Alkaline Phosphatase (80  $\mu$ l)

**Vial 4**

Purified apoB standard

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 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 delipidated apoB is poorly recognized.

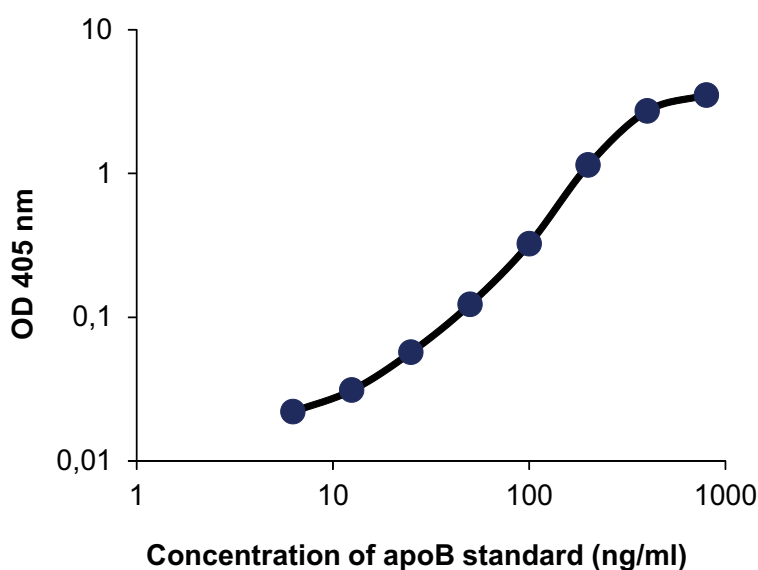
Please note that wash-, block- and incubation buffers should contain detergent. Tween 20, Triton X-100 or NP40 can be used at a concentration of 0.05-0.5%. In block and incubation buffers it is recommended to use 0.1% BSA.

**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 (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. 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.mabtech.com/knowledge-center/apodilution>.

**Reagents:** Antibodies are supplied in sterile-filtered (0.2  $\mu\text{m}$ ) PBS with sodium azide (0.02%). Streptavidin-ALP is supplied in 0.1 M Tris buffer with 0.002% Kathon CG.

**Standard range:** 8-800 ng/ml

**Standard calibration:** No international standard exists for calibration.



# Guidelines for Human Apolipoprotein B ELISA

- Day 1**
1. Coat a high protein binding ELISA plate with mAb LDL 20/17, 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. The apoB standard is supplied as purified LDL stabilised by 50% glycerol. The concentration is 125  $\mu\text{g}/\text{ml}$ . 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. Please note the special considerations for serum/plasma samples described above. Dilution recommendations for serum/plasma samples can be found at <https://www.mabtech.com/knowledge-center/tutorials-and-guidelines/apolipoprotein-dilution>.
  7. Wash as in step 4.
  8. Add 100  $\mu\text{l}/\text{well}$  of mAb LDL 11-biotin at 1  $\mu\text{g}/\text{ml}$  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.



**The products are for research use only.**

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