ELISA for Human Apolipoprotein B

Product Code: 3715-1A-6

CONTENTS, development kit for 6 plates:

Vial 1 (blue)
Monoclonal antibody LDL 20/17 (150 µl)
Concentration: 1 mg/ml

Vial 2 (red top)
Biotinylated monoclonal antibody LDL 11 (80 µl)
Concentration: 1 mg/ml

Vial 3 (white top)
Streptavidin-Alkaline Phosphatase (80 µl)

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 supernatant.

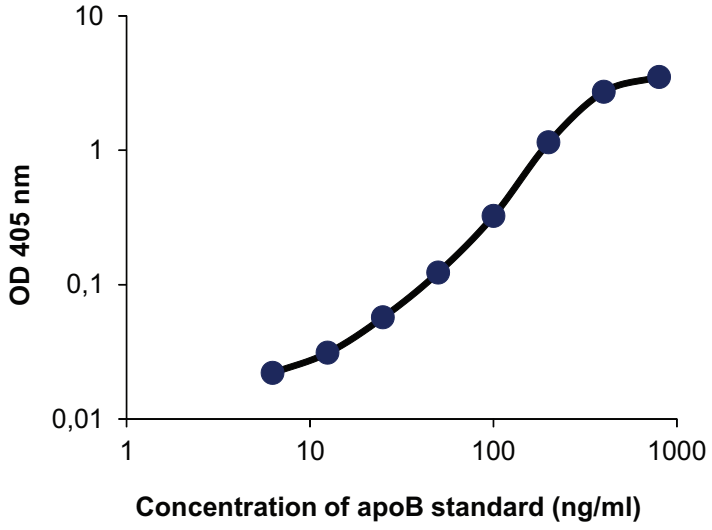
Serum/plasma samples: Please note that determination of analyte in human serum/plasma samples using this kit requires treatment of the samples with Triton X 100, add equal volume of 1% Triton X followed by vortex for 5 seconds. This assay also requires the use of Mabtech Assay buffer (product code: 3652-J2) for dilution of samples, standards and detection antibody. We recommend testing serum/plasma samples at three initial dilutions, e.g. 1:2000, 1:4000, 1:8000. The Assay buffer prevents false positive read-outs which may be caused by interference of heterophilic antibodies commonly found in human plasma and serum. The Assay 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. Please contact Mabtech for further information.

This kit is specific for the detection of apoB100 and does not recognize apoB48. The antibodies do not recognize delipidified apoB. Freezing and thawing plasma will reduce the recognition of apoB by these antibodies.

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 0.15% 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 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.

   Please note the special considerations for serum/plasma samples described above.

5. The apoB standard is supplied as purified LDL stabilised by 50% glycerol. The concentration is 1 mg/ml. 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 Assay buffer for serum/plasma samples Incubate for 1 to 2 hours at room temperature.

7. Wash as in step 4.

8. Add 100 µl/well of mAb LDL 11-biotin at 1 µg/ml in incubation buffer or Assay 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).

13. Measure the optical density (405 nm for pNPP) in an ELISA reader after suitable developing time.

NOTE; for research use only.
MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages therefrom.

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