Human Apolipoprotein B ELISA^{PRO} kit

Kit for 10 plates

Product code: 3715-1HP-10

TABLE OF CONTENTS *PAGE*

KIT DESCRIPTION

Mabtech's enzyme-linked immunosorbent assay, ELISA^{PRO}, is a complete kit for the quantification of human apolipoprotein B (apoB) in biological fluids such as serum, plasma and cell culture supernatants. The assay utilizes ELISA strip plates pre-coated with capture monoclonal antibodies (LDL-20/17), to which samples are added. Captured apoB is detected by adding a biotinylated monoclonal antibody (LDL-11-biotin) followed by streptavidin-horseradish peroxidase (SA-HRP). Addition of the enzyme substrate TMB will result in a colored substrate product with an intensity that is directly proportional to the concentration of apoB in the sample. The concentration of the apoB in the sample is determined by comparison to a serial dilution of purified apoB standard analyzed in parallel. This kit is specific for the detection of apoB100 and does not recognize apoB48. The kit recognizes apoB100 in its form as VLDL/LDL associated protein, whereas purified delipified apoB is poorly recognized.

REAGENTS PROVIDED

Component	Quantity	Description/comments
Precoated 96-well strip plate (12 strips x 8 wells)	10 plates	Plate coated with anti-human apoB (LDL-20/17). Supplied in foil bag with desiccant.
apoB standard	1 vial	Purified LDL in glycerol. See "Preparations" for reconstitution and dilution.
Detection antibody	1 vial (130 µl)	Biotinylated monoclonal antibody, anti-human apoB (LDL-11-biotin) (1 mg/ml). Dilute before use.
SA-HRP	1 vial (130 µl)	Streptavidin-horseradish peroxidase conjugate. Dilute before use.
Triton-X 100 in PBS (ready-to-use)	1 bottle (120 ml)	Sample preparation buffer.
Wash buffer concentrate (20x)	5 bottles (5x120 ml)	For all wash steps. Dilute before use.
Apo ELISA buffer concentrate 5x	3 bottles (2x120 ml, 1x60 ml)	Protein-containing buffer for: 1) dilution of all samples; 2) serial dilution of standard; 3) dilution of detection antibody. Dilute before use.
SA-HRP diluent (ready-to-use)	1 bottle (120 ml)	For dilution of SA-HRP.
TMB substrate (ready-to-use)	1 bottle (120 ml)	3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution containing hydrogen peroxide.
Stop solution (ready-to-use)	1 bottle (120 ml)	0.18 M H_2SO_4
Adhesive plate covers	30	To cover plates during incubations.

To ensure total recovery of stated quantity, bottles and vials are filled with larger volume than indicated.

STORAGE AND EXPIRY DATE

Shipped at ambient temperature. On arrival all components of the kit, with the exception of the purified standard, should be stored at 2-8°C. **Please note that the purified standard should be kept at -20°C.** The expiry date for the unopened kit is indicated on the box. We recommend to use opened kit components within one month.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Microplate reader capable of reading at 450 nm; preferably able of subtracting a reference wavelength between 570-650 nm.
- ELISA plate washer; automatic (adaptable for ELISA strip plates) or manual (e.g. multipipette or squirt bottle).
- Precision pipettes and tips.
- Beakers, flasks and graduated cylinders necessary for reagent preparations.
- Tubes for standard and sample dilutions.
- Distilled or deionized water.

SAFETY ISSUES

- The stop solution, 0.18 M H_2SO_4 (<1%), is irritating to eyes and skin and should be handled with care.
- The standard should be handled with care due to unknown effects of exposure.
- Buffers and reagents in solution contain 0.002% of the preservative Kathon CG, a potential contact allergen which may cause sensitization by skin contact.
- Human and animal samples should be treated as potentially hazardous biological material.
- All material and samples should be disposed of in accordance with local regulations.

PROCEDURAL NOTES

- Do not combine components from different kit lots or components from other suppliers.
- Dilutions of standard and samples can be prepared in plastic or glass tubes.
- Total sample or standard volume added per well should not exceed 100 µl.
- The use of duplicates for each dilution of the standard, samples and blank is recommended.
- Prior to adding new reagents to the wells, ensure that there is no residual wash buffer remaining in the wells. The wells can be emptied by turning the plate upside down followed by gently tapping the plate against clean tissue paper. Please be careful to avoid that the strips fall out of the frame e.g. grip the plate by the middle.
- Serum/plasma components present in the sample may have an impact on the performance of the assay. For this reason, all samples should be diluted at least 2x in "Apo ELISA buffer".
- Sodium azide and other nucleophilic substances (often used as preservative in various buffers) interfere with the activity of horseradish peroxidase. Thus, avoid the use of other wash buffers or solutions that may contain such interfering substances.

PREPARATIONS

Plates
Allow the plates to adjust to room temperature (18-25°C) before opening the bags.

Plan the experiment to include a standard curve and an assay background control (8 x 2 wells), blank (2 wells) and sample wells. To the blank wells, only "TMB substrate" and "Stop solution" should be added. Before the analysis of absorbance values, the mean value of the blank wells should be subtracted from the standard, the assay background control and the sample values.

Assemble the required numbers of strips in the plate frame. The strips used for the experiment can be marked e.g. with a marker pen. Store the remaining strips in the foil bag containing the desiccant at 4-8°C.

Wash buffer
Prepare the required volume of wash buffer by diluting "Wash buffer concentrate" 20x with distilled or deionized water. For 1 plate, prepare 1000 ml wash buffer by adding 50 ml "Wash buffer concentrate" to 950 ml distilled or deionized water.

Apo ELISA buffer
Prepare the required volume of Apo ELISA buffer by diluting "Apo ELISA buffer concentrate 5x" 5x with distilled or deionized water. For 1 plate, prepare 150 ml Apo ELISA buffer by adding 30 ml "Apo ELISA buffer concentrate 5x" to 120 ml distilled or deionized water.

ELISA standard
The apoB standard is supplied as purified LDL stabilised by 50% glycerol. The concentration is 125 µg/ml. It is not necessary to aliquote the standard as the high content of glycerol keeps the standard in a liquid state. Store at -20°C.

Preparation of standard curve
The standard curve should be made from apoB standard stock solution diluted in "Apo ELISA buffer". Prepare a serial dilution of the standard no more than 30 min prior to the experiment. Duplicate wells for the standard are recommended. Dilute the standard stock solution to create a standard curve ranging from 7.8 -500 ng/ml according to the scheme below. For the assay background control (0 pg/ml), use only "Apo ELISA buffer".

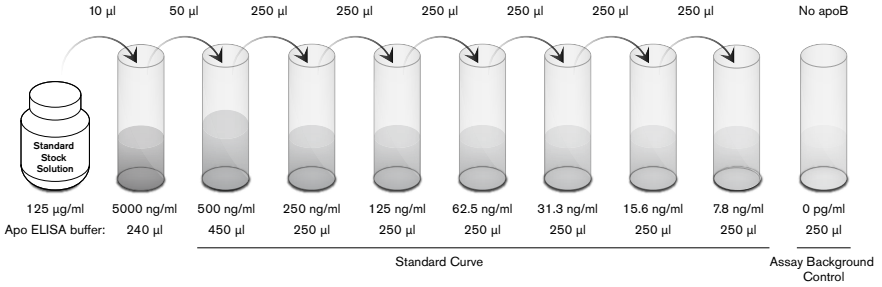


Figure 1. Recommended serial dilution of apoB standard. The volumes indicated are sufficient for duplicates. The last vial should be 0 pg/ml of standard i.e. the analyte should be omitted.

Samples

It is recommended that visible precipitate in the sample should be removed by centrifugation. To prevent interference by different LDL-particle sizes, all serum/plasma derived samples should first be diluted 2x in Triton X-100 followed by vortex for 5 seconds. Such treatment can be done on undiluted or up to 25x diluted serum/plasma samples. Triton X-treatment is not necessary for the apoB standard or for cell line produced samples. Dilute all samples at least 2x in "Apo ELISA buffer" prior to use, also samples where low analyte levels are anticipated. We recommend the use of duplicate wells for all samples. It is recommended to dilute serum/plasma samples 5,000x to 8,000x. Please see dilution guidelines at page 10. Freezing and thawing of serum/plasma samples will reduce signal in this assay.

Detection antibody

Dilute the detection antibody "LDL-11-biotin" 1000x in "Apo ELISA buffer" prior to use. For 1 plate, dilute 12 µl "LDL-11-biotin" in 12 ml "Apo ELISA buffer" which will give a final "LDL-11-biotin" concentration of 1 µg/ml.

SA-HRP

Dilute the "SA-HRP" 1000x in "SA-HRP diluent" prior to use. For 1 plate, dilute 12 µl "SA-HRP" in 12 ml "SA-HRP diluent".

ASSAY PROCEDURE

Throughout the assay all reagents and samples, except the "TMB substrate", should be adjusted to room temperature (18-25°C) prior to use. The "TMB substrate" should preferably be used cold.

1. Assemble the numbers of strips required for the standard curve, assay background control, the blanks and samples in the plate frame. The use of duplicates is recommended.

2. Wash the strips with 5x300 µl/well of diluted wash buffer. The wash buffer should be thoroughly removed in immediate relation to the next step.

3. Add 100 µl/well of each concentration of the diluted apoB standard and assay background control. For the samples, add 100 µl/well of pre-diluted sample. Please note the special considerations for serum/plasma samples described above. Dilution guidelines for serum/plasma samples can be found at page 10. Mix by tapping the plate.
Important! Both the serial dilution of the apoB standard and sample dilutions should be made in "Apo ELISA buffer". Leave the blank wells empty. Cover the plate with adhesive plate cover and incubate at room temperature for 2 h.

4. Wash the wells as in step 2.

5. Add 100 µl per well of the detection antibody LDL-11-biotin, diluted 1000x to a final concentration of 1 µg/ml in "Apo ELISA buffer". Leave the blank wells empty. Cover the plate with adhesive plate cover and incubate at room temp for 60 min.

6. Wash the wells as in step 2.

7. Add 100 µl/well of "SA-HRP" diluted 1000x in "SA-HRP diluent". Leave the blank wells empty. Cover the plate and incubate at room temp for 60 min.

8. Wash the wells as in step 2.

9. Develop by adding 100 µl of "TMB substrate" to all wells (including the blank wells) and incubate at room temp in the dark for 15 min.

10. Stop the color development by adding 100 µl of "Stop solution" to all wells (including the blank wells).

11. Measure the absorbance at 450 nm in a microplate reader within 15 min of the addition of the "Stop solution". If possible, use a reader capable of subtracting a reference wavelength between 570-650 nm.

12. The use of ELISA software utilizing e.g. a 4-parameter curve fitting program is recommended for the data analysis. Subtract the mean absorbance value of the blank from the standard, the assay background control and the sample values prior to creating the standard curve and determining the apoB concentrations in the samples. Note that apoB values obtained should be multiplied with the dilution factor used for each sample.

PERFORMANCE OF THE ASSAY

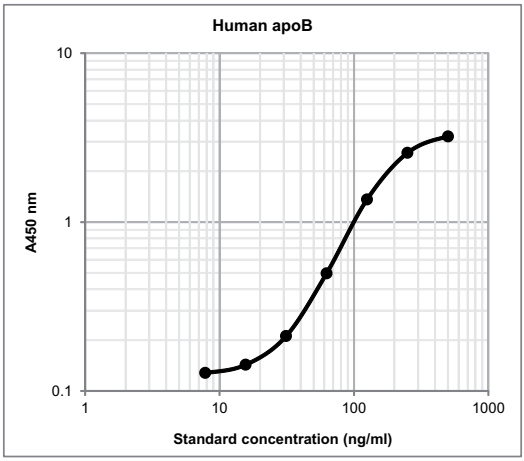


Figure 2. Display of the standard curve.

Standard range: 7.8-500 ng/ml. The standard range is the range in which determinations of analyte concentration can be done with precision, accuracy and linearity.

Sensitivity: The limit of detection of this assay is 7 ng/ml. It is the lowest concentration that is possible to detect but not necessarily quantify with precision and accuracy.

Accuracy: No international standard exists for calibration One ng of supplied standard equals one ng of Triton-X 100 solubilized apoB. Please note that calibration is batch specific.

Dilution recovery: Dilution of serum/plasma samples gives a mid-curve recovery of 90-114% in repeated experiments.

Precision: The intraassay variation is 2.0%(CV). The interassay variation is 10.0%(CV).

LIMITATIONS OF THE ASSAY

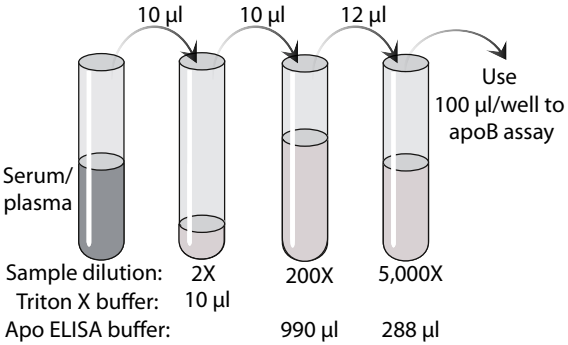
Analysis of samples with high apoB content
The standard curve should not be extrapolated beyond the recommended standard range as these parts of the standard curve are non-linear. Samples yielding absorbance values exceeding the highest point of the standard range should be re-analyzed at a higher dilution.

Heterophilic antibodies in human serum and plasma
Heterophilic antibodies found in human serum/plasma are capable of binding to both the capture and detection antibodies used in capture ELISA. Heterophilic antibodies are found in a majority of human individuals and can, by cross-linking the assay antibodies used, result in false positive signals in capture ELISA. The "Apo ELISA buffer" provided and used for dilution of samples prevents the heterophilic antibodies from cross-linking the capture and detection monoclonal antibodies. The apoB content of serum/plasma samples can therefore be measured without interference by heterophilic antibodies. The lack of interference by heterophilic antibodies in this MABTECH kit has been validated using serum/plasma samples from normal healthy human blood donors. Please note that heterophilic antibody interference in samples from human subjects with various diseases or other conditions have not been assessed.

DILUTION GUIDELINES

Serum and plasma samples containing EDTA, citrate or heparin may be used. Samples should be aliquoted and frozen. For longer storage, -80°C is recommended. Avoid repeated freezing-thawing cycles. Before dilution, it is recommended that visible precipitate in the sample should be removed by centrifugation. Precise pipetting is important! Please change pipet tips between the different steps and ensure thorough mixing. We recommend samples to be assayed in duplicates. Sample dilutions can preferably be made in polypropylene tubes or plates. Prepare the dilutions as near as possible to the start of the assay. The indicated volumes are sufficient for duplicates. The recommended dilutions are based on serum samples from fasting healthy subjects. Samples containing levels of the analyte beyond the standard range will require other dilutions. To prevent interference by different LDL-particle sizes, serum/plasma samples should be treated with Triton X buffer. Triton X treatment, will not interfere with the analysis of other apolipoproteins.

For analysis of human ApoB



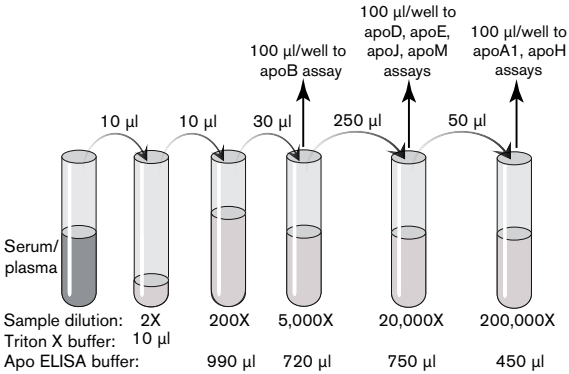
It is recommended to dilute the samples 5,000x to 8,000x. Example of dilution;

1) 10 µl of plasma to 10 µl of Triton X buffer, giving a dilution of 2x, vortex for 5 seconds.

2) 10 µl of 2x dilution to 990 µl Apo ELISA buffer, giving a dilution of 200x.

3) 12 µl of 200x to 288 µl Apo ELISA buffer, giving a dilution of 5,000x

When analyzing several apolipoproteins at the same time



1) 10 µl of plasma to 10 µl of Triton X buffer, giving a dilution of 2x, vortex for 5 seconds.

2) 10 µl of 2x dilution to 990 µl Apo ELISA buffer, giving a dilution of 200x.

3) 30 µl of 200x to 720 µl Apo ELISA buffer, giving a dilution of 5,000x, use 100 µl/well to apoB ELISA

4) 250 µl of 5000x dilution to 750 µl Apo ELISA buffer, giving a dilution of 20,000x, use 100 µl/well to apoE, apoD, apoJ and apoM ELISAs

5) 50 µl of 20000x to 450 µl Apo ELISA buffer giving a dilution of 200,000x, use 100 µl/well to apoA1 and apoH ELISA

MABTECH AB
Augustendalstorget 9
Box 1233, SE-131 28 Nacka Strand
Sweden
Tel: +46 8 716 27 00
Fax: +46 8 716 27 01
E-mail: mabtech@mabtech.com

MABTECH Inc
M.E.B. 220, 3814 West Street
Cincinnati, OH 45227
USA
Toll free: +1 866 ELI-SPOT
Tel: +1 513 871 4500
Fax: +1 513 871 7353
E-mail: mabtech.usa@mabtech.com

MABTECH Australia Pty Ltd
Australia
Tel: +61 3 9470 4704
Fax: +61 3 8678 3216
E-mail: mabtech.au@mabtech.com

MABTECH AB Büro Deutschland
Germany
Tel: +49 40 4135 7935
Fax: +49 40 4135 7945
E-mail: mabtech.de@mabtech.com

MABTECH AB Bureau de liaison
France
Tel: +33 (0)4 92 38 80 70
Fax:+33 (0)4 92 38 80 71
E-mail: mabtech.fr@mabtech.com

MABTECH

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.

ISO 13485:2016 CERTIFICATION TM Intertek

ISO 9001:2015 CERTIFICATION TM Intertek

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

For more information about Mabtech services and our products, visit

www.mabtech.com

Updated on 2019-04-17