ELISA^{PRO} kit for Human apoB

Kit for 2 plates

Product code: 3715-1HP-2

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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 a capture monoclonal antibody (mAb), to which samples are added. Captured apoB is detected by adding a biotinylated mAb 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.

REAGENTS PROVIDED

Component	Quantity	Description/comments
Precoated 96-well strip plate (12 strips x 8 wells)	2 plates	Plate coated with anti-Human apoB. Supplied in foil bag with desiccant.
apoB standard	1 vial	Purified apoB in glycerol. See "Preparations" for reconstitution and dilution.
Detection antibody	1 vial (30 µl)	Biotinylated anti-human apoB mAb (1 mg/ml). Dilute before use.
SA-HRP	1 vial (30 µl)	Streptavidin-horseradish peroxidase conjugate. Dilute before use.
Triton-X 100 in PBS (ready-to-use)	1 bottle (25 ml)	Sample preparation buffer.
Wash buffer concentrate (20x)	1 bottle (120 ml)	For all wash steps. Dilute before use.
ELISA diluent/ Assay buffer (ready-to-use)	1 bottle (120 ml)	Protein-containing buffer for: 1) dilution of all samples; 2) serial dilution of standard; 3) dilution of detection antibody.
SA-HRP diluent (ready-to-use)	1 bottle (25 ml)	For dilution of SA-HRP.
TMB substrate (ready-to-use)	1 bottle (25 ml)	3,3',5,5' tetramethylbenzidine (TMB) enzyme substrate solution containing hydrogen peroxide.
Stop solution (ready-to-use)	1 bottle (25 ml)	1 M H_2SO_4
Adhesive plate covers	6	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.
- PBS with 0.1% BSA for extensive sample dilutions.

SAFETY ISSUES

- The stop solution (1 M H_2SO_4) 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 0.15% 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 "Assay 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.

ELISA standard
The apoB standard is supplied as purified LDL stabilised by 50% glycerol. The concentration is 1 mg/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 "Assay 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 3.16 -3160 ng/ml according to the scheme below. For the assay background control (0 pg/ml), use only "Assay buffer".

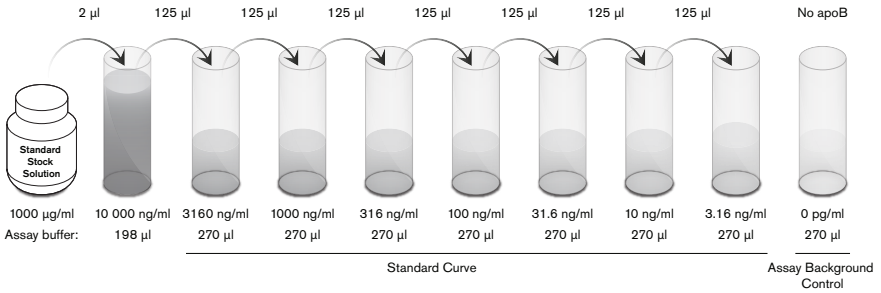

Figure 1. Recommended serial dilution of apoB standard. The volumes indicated are sufficient for duplicates. The last vial should be 0 pg/ml of standard i.e. the analyte should be omitted.

Samples

It is recommended that visible precipitate in the sample should be removed.All serum/plasma derived samples should first be diluted 2x in Triton-X 100 followed by vortex for 5 seconds. This treatment is not necessary for the apoB standard or for cell line produced samples.

Dilute all samples at least 2x in ”Assay buffer” prior to use, also samples where low analyte levels are anticipated. We recommend the use of three dilutions of serum/plasma samples, each in duplicate, e.g. 2000x, 4000x and 8000x. Samples containing high levels of apoB exceeding the standard range of the assay will require further dilution. Initial dilutions can be made in PBS with 0.1% BSA to save ”Assay buffer”. Freezing and thawing of serum/plasma samples will reduce signal in this assay. The antibodies do not recognize delipified apoB.

Detection antibody

Dilute the ”Detection antibody” 1000x in ”Assay buffer” prior to use. For 1 plate, dilute 12 µl ”Detection antibody” in 12 ml ”Assay buffer” which will give a final ”Detection antibody” 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 either 100 µl/well of pre-diluted sample or add 50 µl "Assay buffer" + 50 µl sample per well. Mix by tapping the plate. **Important!** Both the serial dilution of the apoB standard and sample dilutions should be made in "Assay 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 "Detection antibody", diluted 1000x to a final concentration of 1 µg/ml in "Assay 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

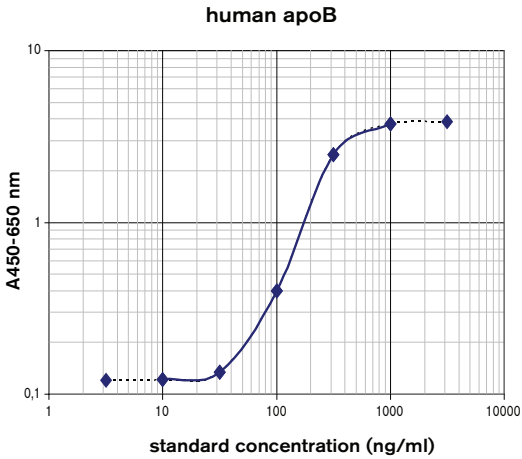

Figure 2. Display of the standard curve.

Standard range: 10-1000 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.

Aberrant sera and plasma
The use of strongly hemolyzed and hyperlipemic serum and plasma samples may result in erroneous determinations of apoB concentrations.

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 "Assay buffer" provided and used for dilution of samples prevents the heterophilic antibodies from cross-linking the capture and detection mAbs. 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.

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