ELISA^{PRO} kit for Human apoE

Kit for 2 plates

Product code: 3712-1HP-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 E (apoE) in biological fluids such as serum, plasma and cell culture supernatants. The three isoforms apoE2, apoE3 and apoE4 are detected. The assay utilizes ELISA strip plates pre-coated with a capture monoclonal antibody (mAb), to which samples are added. Captured apoE 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 apoE in the sample. The concentration of the apoE in the sample is determined by comparison to a serial dilution of recombinant cytokine 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 apoE. Supplied in foil bag with desiccant.
apoE standard	1 vial	Lyophilized recombinant apoE3. See "Preparations" for reconstitution and dilution.
Detection antibody	1 vial (50 µl)	Biotinylated anti-human apoE mAb (0.5 mg/ml). Dilute before use.
SA-HRP	1 vial (30 µl)	Streptavidin-horseradish peroxidase conjugate. Dilute before use.
Standard reconstitution buffer A7 (ready-to-use)	1 vial (1ml)	For reconstitution of lyophilized apoE standard.
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 lyophilized standard, should be stored at 2-8°C. **Please note that the lyophilized standard should be kept at -20°C.** After reconstitution of the lyophilized standard, it should be aliquoted and 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
Reconstitute the apoE standard in 1 ml of standard reconstitution buffer, do not stir. It is important to wait 20 minutes before resuspending the liquid. This gives a stock solution of 5 µg/ml which should be used immediately or stored in aliquots at -20°C for future use. Mix thoroughly and aliquot. Store at -20°C. Avoid repeated freeze-thaw cycles of the standard aliquotes.

Preparation of standard curve
The standard curve can be made from standard stock solution just reconstituted or from thawed aliquotes. 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 0.03-31.6 ng/ml according to the scheme below. For the assay background control (0 pg/ml), use only "Assay buffer".

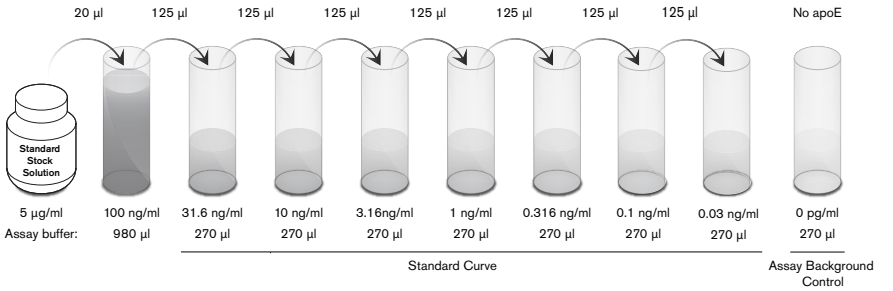

Figure 1. Recommended serial dilution of apoE standard. The volumes indicated are sufficient for duplicates. The last vial should be 0 pg/ml of standard i.e. the analyte should be omitted.

Samples
Dilute all samples in ”Assay buffer” prior to use. Initial dilutions can be made in PBS with 0.1% BSA to save ”Assay buffer”. We recommend the use of duplicate wells for all samples. It is recommended that visible precipitate in the sample should be removed. Serum and plasma samples containing EDTA, citrate or heparin may be used. However, heparin containing samples will give higher apoE values due to displacement of proteoglycan bound apoE.

Important! All samples should be diluted at least 2x in ”Assay buffer”. This applies also for samples where low analyte levels are anticipated. Samples containing high levels of apoE exceeding the standard range of the assay will require further dilution. Sample dilutions can be made either in tubes or directly in the plate. For tube dilutions, equal volumes of ”Assay buffer” and sample should be mixed, e.g. 120 µl ”Assay buffer” + 120 µl sample for a duplicate. If sample dilutions are made directly in the plate, ”Assay buffer” (50 µl per well) should be added prior to addition of the sample (50 µl per well).

Detection antibody
Dilute the ”Detection antibody” 500x in ”Assay buffer” prior to use. For 1 plate, dilute 24 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 apoE standard and assay background control. For the samples, add 100 µl/well of pre-diluted sample. Mix by tapping the plate.
Important! Both the serial dilution of the apoE 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 500x 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 apoE concentrations in the samples. Note that apoE values obtained should be multiplied with the dilution factor used for each sample.

PERFORMANCE OF THE ASSAY

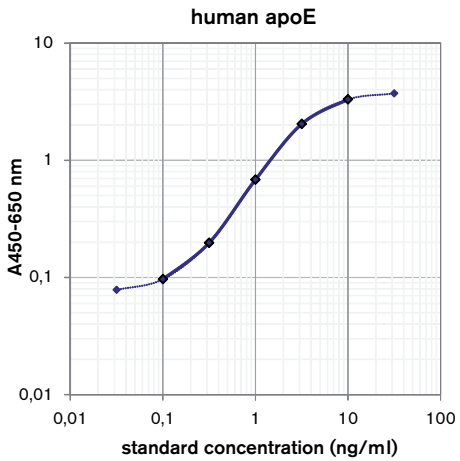

Figure 2. Display of the standard curve.

Standard range: 0.1-10 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 0.02 ng/ml. It is the lowest concentration that is possible to detect but not necessarily quantify with precision and accuracy.

Dilution recovery: Dilution of serum/plasma samples gives a mid-curve recovery of 82-97% in repeated experiments.

Precision: The intraassay variation is 3.0%(CV). The interassay variation is 11.4%(CV).

LIMITATIONS OF THE ASSAY

Analysis of samples with high apoE 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 apoE 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 apoE 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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