Mouse Apolipoprotein A1 ELISA^{PRO} kit

Kit for 2 plates

Product code: 3750-1HP-2

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KIT DESCRIPTION

Mabtech's enzyme-linked immunosorbent assay, ELISA^{PRO}, is a complete kit for the quantification of mouse apolipoprotein A1 (apoA1) in biological fluids such as serum, plasma and cell culture supernatants. The assay utilizes ELISA strip plates pre-coated with a capture monoclonal antibody (mHDL93), to which samples are added. Captured apoA1 is detected by adding a biotinylated monoclonal antibody (mHDL36-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 apoA1 in the sample. The concentration of the apoA1 in the sample is determined by comparison to a serial dilution of purified mouse apoA1 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-mouse apoA1 (mHDL93). Supplied in foil bag with desiccant.
ApoA1 standard	1 vial	Lyophilized purified mouse apoA1. See "Preparations" for reconstitution and dilution
Detection antibody	1 vial (50 µl)	Biotinylated monoclonal antibody, anti-mouse apoA1 (mHDL36) (0.5mg/ml). Dilute before use.
SA-HRP	1 vial (30 µl)	Streptavidin-horseradish peroxidase conjugate. Dilute before use.
Standard reconstitution buffer A8 (ready-to-use)	1 vial (1ml)	For reconstitution of lyophilized mouse apoA1 standard.
Wash buffer concentrate (20x)	1 bottle (120 ml)	For all wash steps. Dilute before use.
5x Sample diluent	1 bottle (60 ml)	Protein-containing buffer for: 1) dilution of all samples; 2) serial dilution of standard. Dilute before use.
Dilution buffer	1 bottle (60 ml)	Protein-containing buffer for: 1) dilution of detection antibody; 2) 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)	0.18 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.

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.

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.
- Components present in the samples may have an impact on the performance of the assay. For this reason, all samples should be diluted at least 2x in "Sample diluent".
- 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.

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

ELISA standard
Reconstitute the mouse apoA1 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 1 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 0.16-10 ng/ml according to the scheme below. For the assay background control (0 ng/ml), use only "Sample diluent".

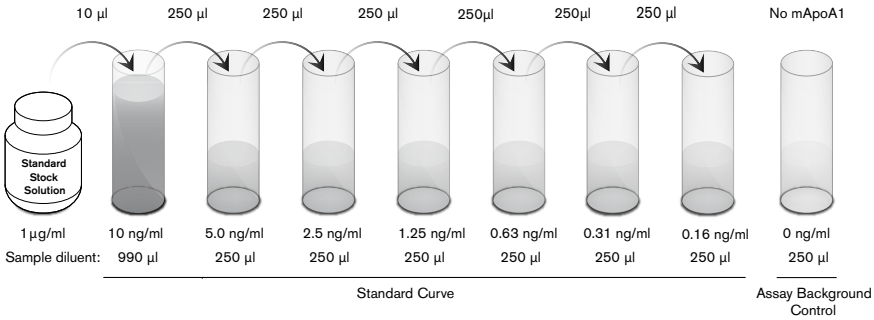


Figure 1. Recommended serial dilution of mouse apoA1 standard. The volumes indicated are sufficient for duplicates. The last vial should be 0 ng/ml of standard i.e. the analyte should be omitted.

Samples

It is recommended that visible precipitate in the sample should be removed by centrifugation. Dilute all samples at least 2x in "Sample diluent" 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 150,000x to 200,000x. Please see dilution guidelines at page 10. Avoid repeated freezing-thawing cycles.

Detection antibody

Dilute the detection antibody "mHDL-36-biotin" 500x in "Dilution buffer" prior to use. For 1 plate, dilute 24 µl "mHDL36-biotin" in 12 ml "Dilution buffer" which will give a final "mHDL36-biotin" concentration of 1 µg/ml.

SA-HRP

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

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. Add 100 µl/well of each concentration of the diluted mouse apoA1 standard and assay background control. For the samples, add 100 µl/well of pre-diluted sample. Dilution guidelines for serum/plasma samples can be found at page 10. Mix by tapping the plate. **Important!** Both the serial dilution of the mouse apoA1 standard and sample dilutions should be made in "Sample diluent". Leave the blank wells empty. Cover the plate with adhesive plate cover and incubate at room temperature for 60 min.

3. 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.

4. Add 100 µl per well of detection antibody mHDL36-biotin, diluted 500x to a final concentration of 1 µg/ml in "Dilution buffer". Leave the blank wells empty. Cover the plate with adhesive plate cover and incubate at room temp for 60 min.

5. Wash the wells as in step 3.

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

7. Wash the wells as in step 3.

8. 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.

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

10. 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.

11. The use of ELISA software utilizing e.g. a 4-or a 5-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 apoA1 concentrations in the samples. Note that apoA1 values obtained should be multiplied with the dilution factor used for each sample.

PERFORMANCE OF THE ASSAY

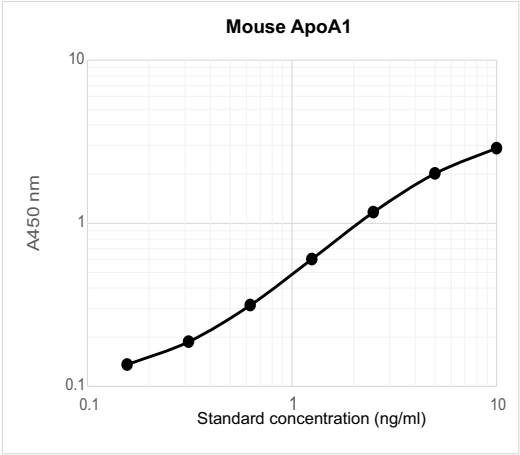


Figure 2. Display of the standard curve.

Standard range: 0.16-10 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 0.03 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 94-107% in repeated experiments.

Precision: The intraassay variation is 3.7%(CV). The interassay variation is 4.5%(CV).

LIMITATIONS OF THE ASSAY

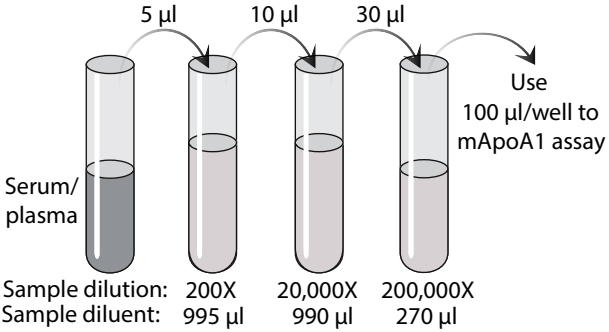
Analysis of samples with high apoA1 content
The standard curve should not be extrapolated beyond the recommended standard range as these parts of the standard curve are non-linear. Samples yielding absorbance values exceeding the highest point of the standard range should be re-analyzed at a higher dilution.

Aberrant sera and plasma
The use of strongly hemolyzed and hyperlipemic serum and plasma samples may result in erroneous determinations of apoA1 concentrations.

DILUTION GUIDELINES

Serum and plasma samples containing EDTA 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 plasma samples from BALB/c and C57BL/6. Samples containing levels of the analyte beyond the standard range will require other dilutions then the recommended ones.

For analysis of mouse ApoA1



It is recommended to dilute the samples 150,000x to 200,000x. Example of dilution;

1) 5 µl of plasma to 995 µl Sample diluent, giving a dilution of 200x.

2) 10 µl 200x dilution to 990 µl Sample diluent, giving a dilution of 20,000x.

3) 30 µl of 20,000x dilution to 270 µl Sample diluent, giving a dilution of 200,000x.

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