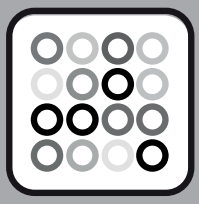
MABTECH

ELISA Pro: Human ApoB

3715-1HP-1 | 3715-1HP-2 | 3715-1HP-10

Datasheet & Protocol



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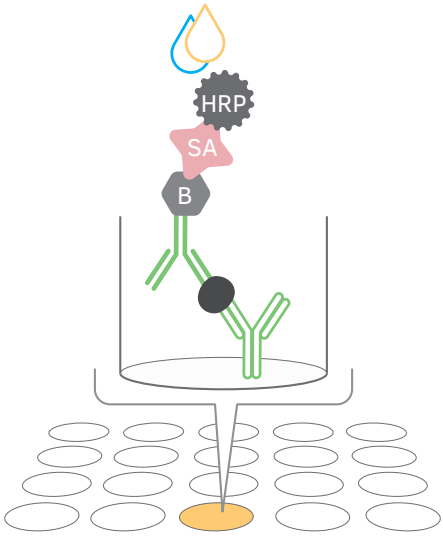
Introduction

Mabtech's carefully validated ELISA Pro kits provide all the necessary reagents to conveniently quantify analytes in serum, plasma, and cell culture supernatants in a robust, sensitive, and specific manner. For Research Use Only (RUO). Not for use in diagnostic procedures.

ELISA assay principle

ELISA Pro kits are supplied with ELISA strip plates precoated with monoclonal antibody (mAb). Analyte in the sample is captured by the coated mAb and detected by the biotinylated detection mAb followed by Streptavidin-HRP (SA-HRP). Addition of TMB substrate will result in a colored substrate product. The reaction is stopped with sulfuric acid and the optical density can be quantified using an ELISA plate reader. The concentration of analyte is determined by comparison to a serial dilution of the ELISA standard analyzed in parallel.



Analysis of serum and plasma samples

The ELISA Pro kits include Apo ELISA buffer, a buffer that prevents false-positive signals. The buffer blocks heterophilic antibodies from cross-linking the assay antibodies. Heterophilic antibodies are commonly found in human serum/plasma and can also be present in other species. The buffer has been validated using serum/plasma samples from healthy human blood donors.

Shipping and storage

The kit is shipped at ambient temperature. All reagents should be stored at 4-8 °C upon receipt, except the standard, which should be stored at -20 °C. The expiry date indicates how long unopened products, stored according to instructions, are recommended for use. Do not combine components from different kit batches or components from other suppliers.

Contents

Component	1-plate kit	2-plate kit	10-plate kit
Pre-coated ELISA strip plate: Anti-apoB mAbs LDL 20/17	1 x 96 wells	2 x 96 wells	10 x 96 wells
Human apoB ELISA standard: Purified LDL in glycerol	1 vial	1 vial	1 vial
Detection mAb LDL 11, biotin (1 mg/ml)	15 µl	25 µl	125 µl
Streptavidin-HRP	15 µl	25 µl	125 µl
Wash buffer concentrate	120 ml	120 ml	5 x 120 ml
Apo ELISA buffer concentrate 5x	60 ml	60 ml	5 x 60 ml
Streptavidin-HRP diluent	15 ml	25 ml	120 ml
Triton-X in PBS	15 ml	25 ml	125 ml
TMB substrate	15 ml	25 ml	120 ml
Stop solution	15 ml	25 ml	120 ml
Adhesive plate covers	3	6	30

To ensure total recovery of the stated quantity, bottles and vials have been overfilled.

Materials required but not supplied

- Microplate reader capable of reading at 450 nm
- ELISA plate washer; automated or manual (e.g., multipipette or squirt bottle)
- Precision pipettes, tips, and graduated cylinders
- Tubes for standard and sample dilutions
- Distilled or deionized water

Safety information

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 also be handled carefully as the effects of exposure are unknown. Buffers and reagents in solution contain the preservative Kathon CG (0.002%), a potential allergen that may cause sensitization through skin contact. Human and animal samples should be treated as potentially hazardous biologic material. All material should be disposed of in accordance with local regulations. For further information please consult the Safety Data Sheet on our website.

Preparation

- Allow the plates and assay reagents to reach room temperature before starting the assay (except for the TMB substrate which should preferably be used cold).
- Plan the plate layout to include a standard curve, samples, and an assay background control, all in duplicate. The volume per well should not exceed 100 µl. Include a plate blank (wells with only Substrate and Stop solution) to be used for subtraction before analysis.

Wash buffer

Add 50 ml Wash buffer concentrate to 950 ml distilled or deionized water (sufficient for all washing steps of 1 plate). If crystals have formed in the 20x concentrate, bring to room temperature and mix gently to dissolve.

Apo ELISA buffer

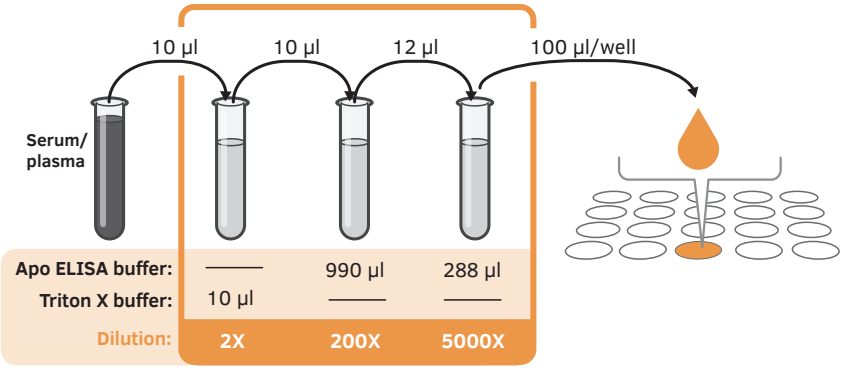
Prepare the required volume of Apo ELISA buffer by diluting Apo ELISA buffer concentrate 5-fold with distilled or deionized water. For each plate, add 30 ml Apo ELISA buffer concentrate to 120 ml water.

Samples

All samples should be diluted at least 2-fold in Apo ELISA buffer. Remove visible precipitates and dilute in tubes/plates, buffer should be added prior to the samples. Strongly hemolyzed and hyperlipemic samples may give inaccurate quantifications. To prevent interference by different LDL particle sizes, all serum/plasma derived samples should be diluted 2-fold in Triton X-100 buffer, followed by vortex for 5 sec. Triton X dilution is not necessary for cell-line produced samples, and will not interfere with analysis of other apolipoproteins. Avoid freeze-thaw cycles as it will lead to lower signals.

Dilution guidelines for human serum/plasma

We recommend a dilution factor of 5000X based on repeated analyses of fasting healthy subjects. Precise pipetting is important, change tips between dilution steps and use freshly made dilutions. Indicated volumes are sufficient for duplicates.

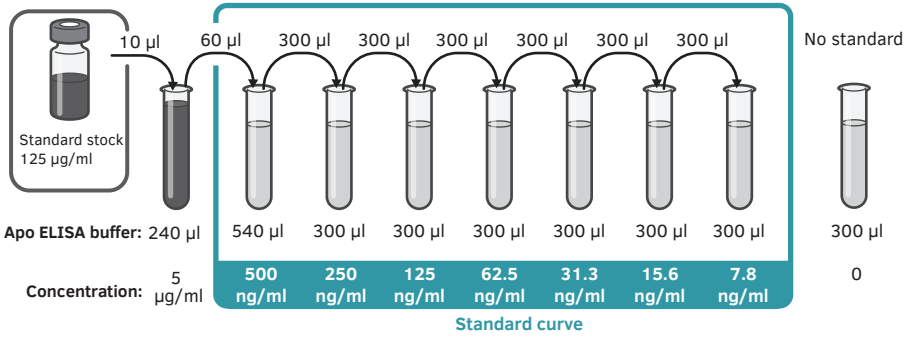


ELISA standard

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

Dilute the standard stock solution to create a standard curve as shown. The indicated volumes are sufficient for duplicates. The last vial is used as an assay background control, i.e., the standard should be omitted. Prepare the standard curve within 30 minutes of use.



Detection antibody

Dilute the detection mAb in Apo ELISA buffer to a concentration of 1 µg/ml within 15 minutes of use. For each plate, add 12 µl detection mAb to 12 ml Apo ELISA buffer.

Streptavidin-HRP

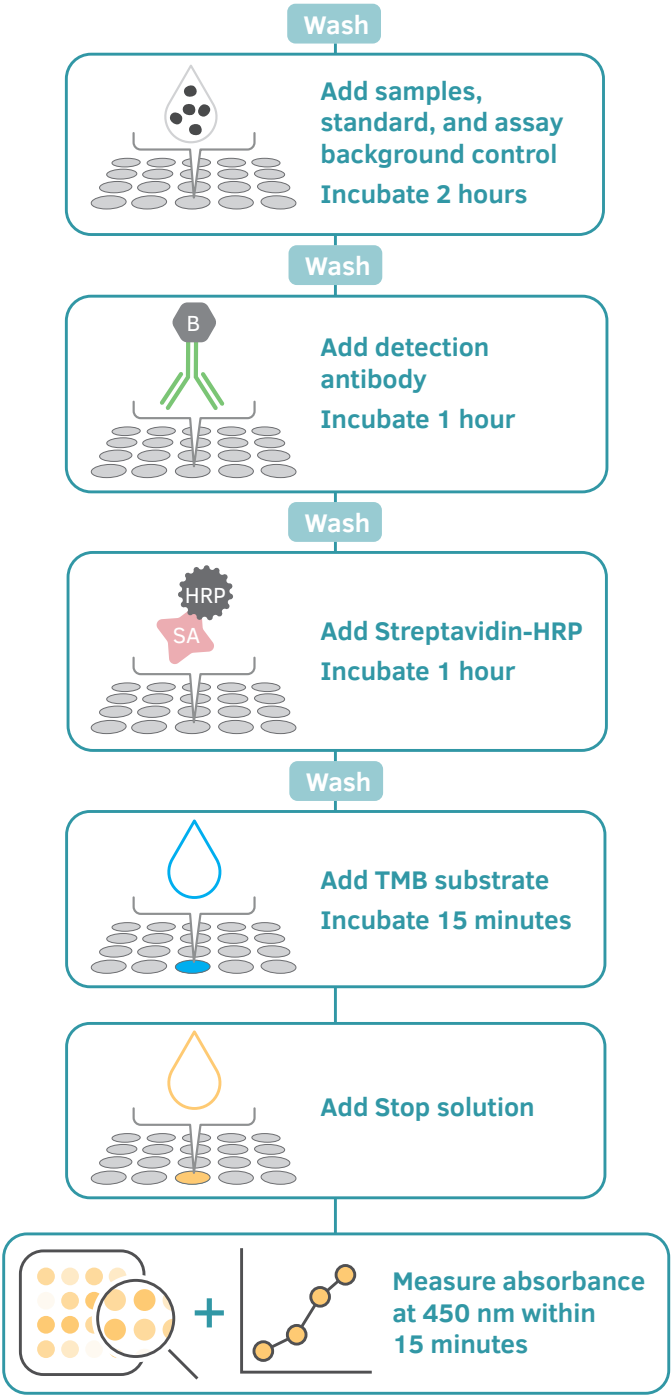
Dilute the Streptavidin-HRP 1:1000 in Streptavidin-HRP diluent within 15 minutes of use. For each plate, add 12 µl Streptavidin-HRP to 12 ml Streptavidin-HRP diluent.

Protocol

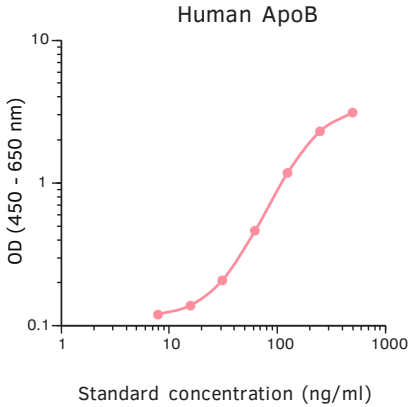
Prepare the reagents, standard curve, and samples as described in the Preparation section. Assemble the required number of strips in the plate frame and label the top of each strip. Store the remaining strips in the foil bag containing the desiccant at 4-8 °C.

1. Wash the plate 5 times with wash buffer, 300 µl per well. After the final wash, invert and tap the plate firmly against absorbent paper. Immediately proceed to the next step.
2. Add 100 µl per well of samples (diluted at least 2-fold), standard, and assay background control. Mix by tapping the plate. Cover the plate with an adhesive plate cover and incubate for 2 hours at room temperature.
3. Wash as in step 1.
4. Add 100 µl per well of detection mAb. Cover the plate and incubate for 1 hour at room temperature.
5. Wash as in step 1.
6. Add 100 µl per well of Streptavidin-HRP. Cover the plate and incubate for 1 hour at room temperature.
7. Wash as in step 1.
8. Add 100 µl of TMB substrate to each well. Incubate at room temperature, protected from direct light for 15 minutes.
9. Add 100 µl of Stop solution to each well to stop the color development.
10. Measure absorbance at 450 nm within 15 minutes. Preferably use a reader capable of subtracting a reference wavelength between 570 and 650 nm.

    We recommend the use of an ELISA software utilizing a 4- or 5-parameter curve fit. Subtract the mean absorbance value of the blank from the samples, standard and assay background control prior to creating the standard curve and analyzing the results.


Wash
Add samples, standard, and assay background control
Incubate 2 hours
Wash
B
Add detection antibody
Incubate 1 hour
Wash
HRP
SA
Add Streptavidin-HRP
Incubate 1 hour
Wash
Add TMB substrate
Incubate 15 minutes
Add Stop solution
Measure absorbance at 450 nm within 15 minutes

Performance



Representative standard curve

Standard range 7.8-500 ng/ml

Sensitivity 7 ng/ml

The lowest concentration that can be detected, but not necessarily quantified with precision and accuracy. This was determined by adding 2 standard deviations to the mean OD of background wells.

Calibration

No international standard exists for calibration of Triton-X 100 solubilized apoB. Please note that calibration is batch specific.

Precision

	Intra-assay		Inter-assay	
Sample	1	2	1	2
n	8	8	6	6
Mean (ng/ml)	268.3	146.8	261.0	136.6
SD	8.5	3.0	26.2	14.8
CV%	3.2	2.0	10.0	10.8

Intra-assay and inter-assay precision were determined at 2 different concentrations of analyte (8 replicates per concentration in 6 assays).

Linearity

Dilution of a human plasma gives a mid-curve recovery of 90-114% in repeated experiments (mean 99%).

Specificity

The kit is based on a matched pair of mAbs specific for human ApoB100. The antibodies cross-react with apoB from non-human primates. Please visit www.mabtech.com for reactivity on NHP species.

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MABTECH shall not be liable for the use or handling of the product or for consequential, special, indirect or incidental damages there from.

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