Amplite™ Human Apolipoprotein M (ApoM) Kit *Optimized For ELISAPro Automated ELISA Processing*

Catalog number: V101065
Unit size: 96 Tests

Component	Storage	Amount
Standard reconstitution buffer A8	Refrigerated (2-8 °C), Minimize light exposure	1 vial (1 mL)
Adhesive plate covers	Refrigerated (2-8 °C), Minimize light exposure	3
Apo ELISA buffer concentrate 5x	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (60 mL)
Biotinylated detection mAb M7	Refrigerated (2-8 °C), Minimize light exposure	1 vial (15 uL, 0.5 mg/mL)
Pre-coated ELISA strip plate (Anti-apoM mAb M5)	Refrigerated (2-8 °C), Minimize light exposure	1x96 wells
Purified human apoM ELISA standard	Freeze (< -15 °C), Minimize light exposure	1 vial
Stop solution	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (15 mL)
Streptavidin-HRP (1000x)	Refrigerated (2-8 °C), Minimize light exposure	1 vial (15 uL)
Streptavidin-HRP diluent	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (15 mL)
TMB substrate	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (15 mL)
Wash buffer concentrate (20X)	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (120 mL)

AT A GLANCE

ELISAPRO kits provide all the necessary reagents to conveniently quantify analytes in serum, plasma, and cell culture supernatants in a robust, sensitive, and specific manner.

ELISA assay principle
ELISAPRO 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 ELISAPRO 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.

Materials required but not supplied

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

Safety information
The Stop solution, 0.18 M H2SO4 (< 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. Plate blanks (only substrate and Stop solution) can be included for subtraction by the reader software.

KEY PARAMETERS

Absorbance microplate reader

Absorbance	450 nm
Recommended plate	Clear bottom

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. 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.

2. 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.

3. Samples
All samples should be diluted at least 2-fold in Apo ELISA buffer. Remove visible precipitates and dilute in polypropylene tubes/plates, buffer should be added prior to the samples. Strongly hemolyzed and hyperlipemic samples may result in inaccurate quantifications. Samples containing high levels of analyte exceeding the standard range of the assay will require further dilution.

4. Dilution guidelines for human serum/plasma
We recommend a dilution factor of 20,000X 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.

5. ELISA standard
Reconstitute the ELISA standard to a stock solution of 0.5 µg/ml by adding 260 µl Standard reconstitution buffer. Do not stir. Allow the standard to dissolve for 20 minutes and mix thoroughly. The standard should be kept in aliquots at -20 °C. Avoid repeated freeze-thaw cycles.

6. 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.

PREPARATION OF WORKING SOLUTION

1. Detection antibody
Dilute the detection antibody in Apo ELISA buffer to a concentration of 0.5 µg/ml within 15 minutes of use. For each plate, dilute 12 µl detection antibody in 12 ml Apo ELISA buffer.

2. Streptavidin-HRP
Dilute the Streptavidin-HRP 1000-fold in Streptavidin-HRP diluent within 15 minutes of use. For each plate, dilute 12 µl Streptavidin-HRP in 12 ml Streptavidin-HRP diluent.

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

EXAMPLE DATA ANALYSIS AND FIGURES

Example data analysis and images of this product can be found on the web at:
https://www.aatbio.com/products/amplite-human-apolipoprotein-m-apom-kit-optimized-for-elisapro-automated-elisa-processing

Standard range
0.027-20 ng/ml

Sensitivity 0.01 ng/ml
The lowest concentration that can be detected, but not necessarily quantified with precision and accuracy. This was determined by adding 5 standard deviations to the mean OD of background wells.

Calibration
No international standard exists for calibration.

Specificity
The ELISAPRO kit contains a matched pair of mAbs specific for human ApoM.

DISCLAIMER