Human Apolipoprotein M ELISA

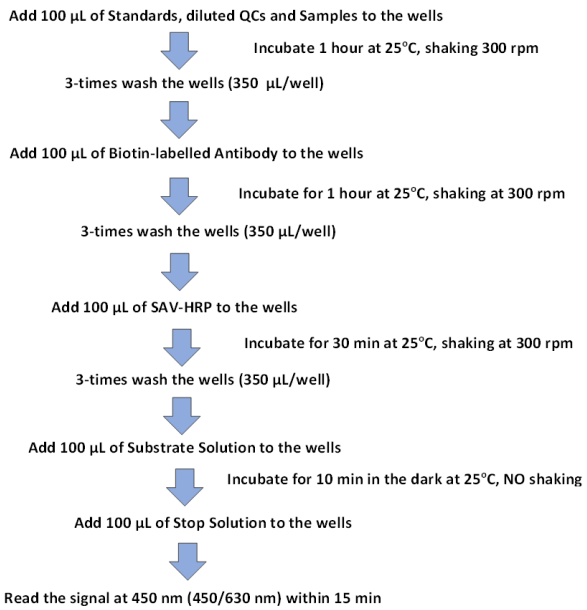
Cat. No.: BA3011

Enzyme Immunoassay for the quantitative determination of Apolipoprotein M (ApoM, Apo M) in human serum and plasma.

Apolipoprotein M is a secreted 25kDa member of the lipocalin protein family. Apo M is predominantly expressed in the mature liver and kidney and plays a role in lipid metabolism. It is found associated with high density lipoproteins (HDL) in human plasma and to a lesser extent with low density lipoproteins (LDL), triglyceride-rich lipoproteins (TGLRP) and chylomicrons and is involved in lipid transport. It plays an important role in the anti-atherogenic function of HDL by influencing the accumulation of cholesterol in these particles and reverse cholesterol transport. ApoM was found to increase the capacity of HDL to induce cholesterol efflux from macrophage foam cells and to inhibit LDL oxidation. ApoM also might be a useful biomarker for predicting the progression of diabetic nephropathy, and the ApoM/S1P-S1P1 axis might serve as a novel therapeutic target for preventing the development/progression of diabetic nephropathy.

PRINCIPLE OF APO M ELISA

The microtiter plate is coated with the antibody specifically binding the Apolipoprotein M. The human serum or plasma is incubated in the plate with the capture antibody.
The specimen is washed out and the specifically bound protein is incubated with biotin-labelled detection antibody. Following another washing step, Streptavidin-HRP conjugate is added into the well.
Unbound reagent is then washed out. Horseradish peroxidase (HRP) bound in the complex reacts with the chromogenic substrate (TMB) creating the blue colour. The reaction is stopped by addition of STOP solution (H_2SO_4).
The absorbance values are measured at 450 nm (optionally 450/630 nm) and are proportional to the concentration of Apo M in the specimen. The concentration of Apo M in unknown samples is determined from the calibration curve which is created by plotting the absorbance values against the standard concentration values.



Kit Contents

Item	***Qty.***
Antibody Coated Microtiter Plate	96 wells
Biotin-labelled Antibody	13 mL
Streptavidin-HRP Conjugate	13 mL
Master Standard (lyophilized)	1 vial
Quality Control A (human serum, lyophilized)	1 vial
Quality Control B (human serum, lyophilized)	1 vial
Dilution Buffer 5x conc.	13 mL
Wash Buffer 15x conc.	50 mL
Substrate Solution	13 mL
STOP Solution	13 mL

MATERIAL REQUIRED BUT NOT SUPPLIED

1. Glassware and test tubes
2. Microtiter plate washer
3. Precision pipettes (various volumes) with tips
4. Orbital shaker
5. Microtiter plate reader capable of measuring absorbance at 450 nm or 450/630 nm with software for data generation

WARNINGS AND PRECAUTIONS

1. For research use only
2. For professional laboratory use
3. The reagents with different lot numbers should not be mixed
4. To prevent cross sample contamination, use disposable labware and pipette tips
5. To protect laboratory stuff, wear protective gloves and protective clothing
6. The substrate solution should remain colourless, keep it protected from light
7. The test should be performed at standard laboratory conditions (temperature 25°C ± 2°C).

STORAGE CONDITIONS

1. The kit must be stored at 2 – 8°C.
2. The opened components can be stored for one week at 2 – 8°C.

PREPARATION OF REAGENTS

- Use new pipette tip for pipetting different reagents and samples to prevent cross-contamination.
- All reagents and samples should be allowed to reach the temperature 25°C ± 2°C.

Preparation of Standards

Reconstitute lyophilized Human APO M Standard in Dilution Buffer, for the volume information see the Certificate of Analysis. Let it rehydrate for 15 min prior to use. The concentration of human APO M in Master Standard is 10 ng/mL.
Prepare set of Standard solution as follows: Use the Master Standard for serial dilution (as below). Mix each tube thoroughly before the next transfer. The Dilution Buffer serves as Blank.

	Volume of Standard	Dilution Buffer	Concentration
Std1	Standard 10 ng/mL (lyophilized)	see CoA	10 ng/mL
Std2	300 µL of Std1	300 µL	5 ng/mL
Std3	300 µL of Std2	300 µL	2.5 ng/mL
Std4	300 µL of Std3	300 µL	1.25 ng/mL
Std5	300 µL of Std4	300 µL	0.625 ng/mL
Std6	300 µL of Std5	300 µL	0.313 ng/mL
Blank	-	300 µL	0 ng/mL

Preparation of Quality Control A and B

Reconstitute the lyophilized human serum Quality Controls in deionized/distilled water, for the volume information see the Certificate of Analysis. Let the QCs rehydrate for 15 min prior to use and dilute them 1:1 000 with Dilution Buffer, see Preparation of samples.

Preparation of Wash Buffer 1x

Prepare a working solution of Wash Buffer by adding 50 mL of Wash Buffer 15x conc. to 700 mL of deionized/ distilled water (dH_2O). Mix well. Store at 4°C for two weeks or at -20°C for long term storage.

Preparation of Dilution Buffer 1x

Prepare a working solution of Dilution Buffer by mixing 13 mL of Dilution Buffer 5x conc. and 52 mL of deionized/ distilled water (dH_2O). Prepare only the amount for immediate consumption. Mix well. Store at 4°C for two weeks.

Preparation of samples

Human serum or plasma may be used with this assay. For long-term storage the samples should be frozen at minimum -70°C. Lipemic or haemolytic samples may cause false results.

Recommended dilution of samples is 1:1000. It is recommended to use the two-step dilution.
Dilution A (50x): 5 µL of samples + 245 µL of Dilution Buffer.
Dilution B (20x): 15 µL of Dilution A + 285 µL of Dilution Buffer.

Do not store the diluted samples.

ASSAY PROCEDURE

1. Prepare the reagents as described in the previous chapter.
2. Pipette 100 µL of set of Standards, diluted Samples and Dilution Buffer = Blank into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
3. Wash the wells 3-times with 1x Wash Buffer (350 µL/well). When finished, tap the plate against the paper towel to remove the liquid completely.
4. Pipette 100 µL of Biotin-labelled Antibody into each well. Incubate for **1 hour** at 25°C ±2°C, shaking at 300 rpm.
5. Wash the wells as described in point 3.
6. Pipette 100 µL of Streptavidin-HRP into each well. Incubate for **30 min** at 25°C ±2°C, shaking at 300 rpm.
7. Wash the wells as described in point 3.
8. Pipette 100 µL Substrate solution, incubate for **10 min**, at 25°C ±2°C. Avoid exposure to the light during this step.
9. Pipette 100 µL of STOP solution.
10. Read the signal at 450 or 450/630 nm within 15 min.

PERFORMANCE CHARACTERISTICS

Samples used in the tests were diluted 1:1000 as recommended and assayed. The results are multiplied by the dilution factor.

1. Sensitivity

The limit of detection, defined as a concentration of human Apo M giving absorbance higher than absorbance of blank + 3 standard deviations, is better than 0.07 ng/mL of sample.

2. Precision

Intra-assay

Sample	Mean (µg/mL)	SD	CV (%)
1	1.94	0.09	4.9
2	3.94	0.19	5.2

Inter-assay (Run – to – run)

Sample	Mean (µg/mL)	SD	CV (%)
1	1.80	0.10	5.7
2	3.96	0.23	5.8

3. Accuracy

Dilution linearity

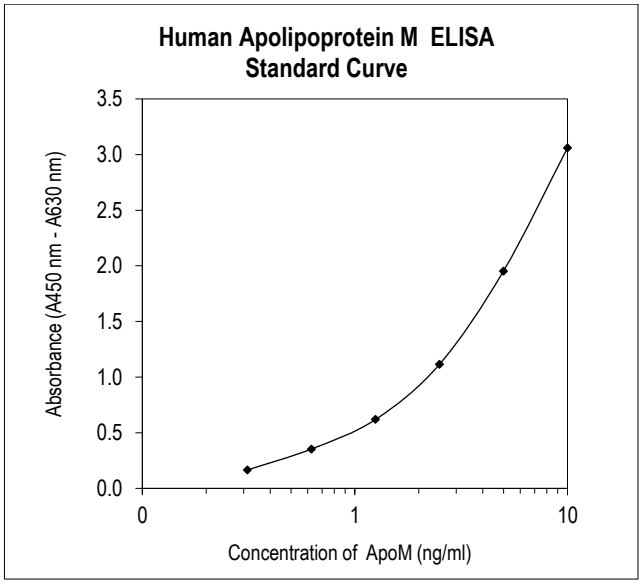
Sample	Dilution	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
1		5.21	-	-
	2x	2.70	2.61	104
	4x	1.44	1.30	110
	8x	0.72	0.65	111
2		4.44	-	-
	2x	2.22	2.21	103
	4x	1.17	1.11	105
	8x	0.64	0.55	115

Spiking Recovery

Sample	Spike (ng/ml)	Measured concentration (µg/mL)	Expected concentration (µg/mL)	Yield (%)
1	-	0.86	-	-
	0.31	1.17	1.17	100
	1.25	1.92	2.11	91
	5.00	4.94	5.86	84

Typical standard curve

The standard curve needs to be measured in every test. Most of the microplate reader can automatically calculate the analyte concentration using 4-parameter algorithm or alternative functions to fit the standard points properly. The concentrations need to be multiplied by the dilution factor, either automatically by reader or manually.



RESOURCES

[1] Xu N, Dahlback B: A novel human apolipoprotein M (apoM). J Biol Chem 1999, 274:31286-31290

[2] Yao Mattison I, Christoffersen C: Apolipoprotein M and its impact on endothelial dysfunction and inflammation in the cardiovascular system. Atherosclerosis. 2021 Oct;334:76-84