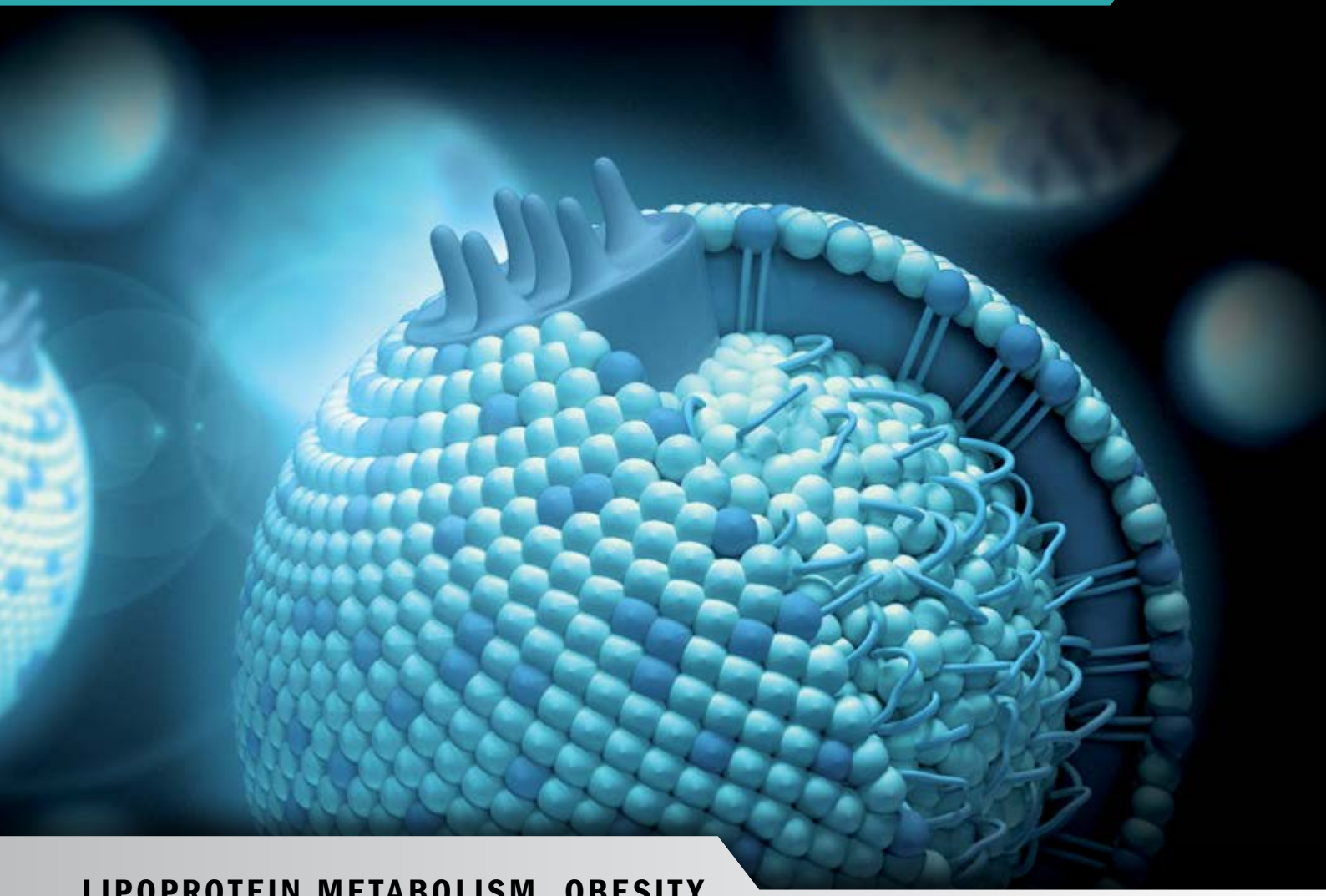
QUANTITATIVE DETERMINATION OF HUMAN APOLIPOPROTEIN M (APO M)

NEW PRODUCT

Human Apolipoprotein M ELISA

- High sensitivity (0.07 ng/ml)
- Excellent analytical characteristics
- Validated for human serum and plasma (EDTA, citrate, heparin) samples



LIPOPROTEIN METABOLISM, OBESITY
ATHEROSCLEROSIS
DIABETES MELLITUS
IMMUNE RESPONSE
INFECTION AND INFLAMMATION

BioVendor
Research
and Diagnostic Products

HUMAN APOLIPOPROTEIN M ELISA

Introduction

Human Apolipoprotein M (ApoM, protein G3a, NG20-like protein NG20), is a protein that in humans is encoded by the APOM gene. Two different isoforms have been found for this gene. ApoM is a secreted 25kDa member of the lipocalin protein family consisting of 188 amino acids. Human APOM shares sequence identity with pig (91%) and mouse (80%) APOM.

The apolipoproteins are a structurally-unrelated group of proteins that all have some role in the transport of lipids in blood. Apolipoproteins plus phospholipids, cholesterol and triglycerides, form spherical particles with a lipid/hydrophobic center and a (apolipo)protein coat. The apolipoprotein coat promotes aqueous solubility and serves as a ligand for lipoprotein receptors. HDL may contain apoliporoteins A,C, D,E, J, L and M, while LDL contains apolipoproteins B and E.

ApoM is only expressed in the adult liver and in kidney and small amounts are found in fetal liver and kidney. ApoM mRNA hybridization demonstrates that apoM is exclusively expressed in the hepatocytes and in the renal tubular epithelium.

ApoM contains an uncleaved, hydrophobic N-terminal segment and one utilized N-linked glycosylation site. 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 (TGRLP) and chylomicrons. The encoded protein is secreted through the plasma membrane but remains membrane-bound, where it is involved in lipid transport.

The uncleaved signal peptide on mature ApoM serves as a hydrophobic anchor. This suggest that apolipoprotein M 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 inhibit LDL oxidation and to increase the capacity of HDL to induce cholesterol efflux from macrophage foam cells by promoting the formation preβ-HDL, an important initial extracellular acceptor of cell-derived cholesterol.

Kidney derived ApoM binds to megalin, a member of the low-density lipoprotein-receptor family, which interacts with many lipocalins in renal tubuli.

Expression of ApoM appears to be regulated by hepatocyte nuclear factor-1α (HNF-1α), platelet activating factor (PAF), transforming growth factor (TGF), insulin-like growth factor (IGF), leptin and leptin receptor. Both, leptin and leptin receptor are essential for ApoM expression.

Additionally, subjects with mutations in HNF-1α (MODY3) have reduced serum ApoM levels compared to controls. Together, these findings suggest that ApoM is related to the initiation and progression of MODY3 and obesity. The results of several studies indicated that ApoM concentration in plasma is positively related to leptin and negatively related to cholesterol levels in humans. However, the most recent studies show that the strongest positive correlation was to ApoA1, total cholesterol and LDL cholesterol. Weaker, but significant, correlations were observed among ApoM, ApoB and HDL

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cholesterol. Thus, ApoM may have a specific function which is related to hepatic lipid and lipoprotein metabolism and it may play important role in the prevention of **atherosclerosis**.

ApoM may also play a role in host defense response because the APOM gene is located in the histocompatibility complex III (HMC-III) region on chromosome 6. Many genes in this region are related to the **immune response system**, and the APOM gene is very close to the TNF-α gene and lymphotoxin genes.

ApoM binds HDL-associated sfingosine-1-phosphate (S1P), a sphingolipid important for vascular barrier protection. ApoM concentrations correlated negatively to acute-phase markers HBP (heparin-binding protein), procalcitonin, CRP and IL-6. During sepsis and SIRS, the plasma concentrations of ApoM decrease with, the degree of decrease reflecting the severity of the disease. As a carrier for barrier protective S1P in HDL, the decrease of ApoM could contribute to the increased vascular leakage observed in sepsis and SIRS.

BioVendor Human Apolipoprotein M ELISA (RD191129200R)

Intended use

The RD191129200R Human Apolipoprotein M ELISA is a sandwich enzyme immunoassay for the quantitative measurement of human Apolipoprotein M.

- The total assay time is less than 3 hours
- The kit measures total apolipoprotein M in serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant protein
- Components of the kit are provided ready to use, concentrated or lyophilized

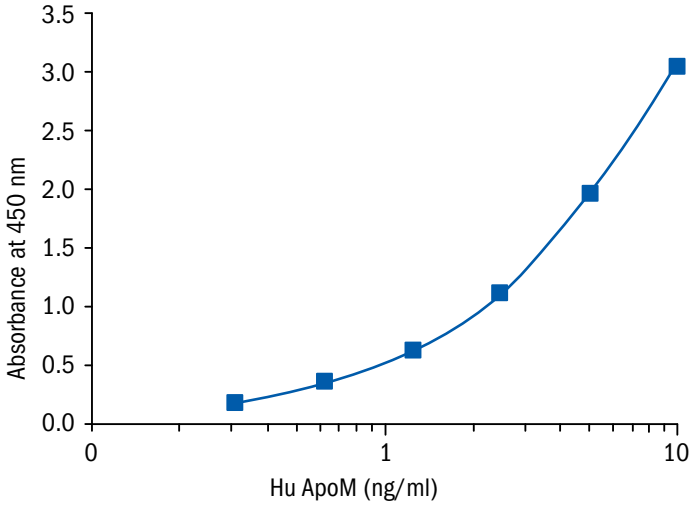
Clinical application

- Lipoprotein metabolism, Obesity
- Atherosclerosis
- Diabetes mellitus
- Immune response, infection and inflammation

HUMAN APOLIPOPROTEIN M ELISA CAT. NO.: RD191129200R	
Assay format	Sanwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Serum, Plasma (EDTA, citrate, heparin)
Standards	0.313 to 10 ng/ml
Limit of detection	0.07 ng/ml

Test principle

In the BioVendor Human Apolipoprotein M ELISA, standards, quality controls and samples are incubated in a microtitrate plate wells pre-coated with polyclonal anti-human apolipoprotein M antibody. After 60 min incubation and a washing, biotin-labelled polyclonal anti-human ApoM antibody is added and incubated with captured ApoM for 60 min. After another washing, the streptavidin-HRP conjugate is added. After 30 min incubation and the last washing step, the remaining conjugate is allowed to react with the substrate solution (TMB). The reaction is stopped by addition of acidic solution and absorbance of the resulting yellow product is measured. The absorbance is proportional to the concentration of Apolipoprotein M. A standard curve is constructed by plotting absorbance values against concentrations of Apolipoprotein M standards, and concentrations of unknown samples are determined using this standard curve.



Precision

Intra-assay (Within-Run) (n=8)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	1939.6	89.6	4.9
2	3935.8	192.1	5.2

Inter-assay (Run-to-Run) (n=5)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	1804.1	102.7	5.7
2	3961.8	229.0	5.8

Spiking recovery

Serum samples were spiked with different amounts of human ApoM, diluted with Dilution Buffer 1000x and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	856.8	-	-
	1173.9	1169.8	100.3
	1920.4	2106.8	91.2
	4936.5	5856.8	84.3
2	2101.5	-	-
	2486.6	2414.5	103.0
	3046.0	3351.5	90.9
	5895.4	7101.5	83.0

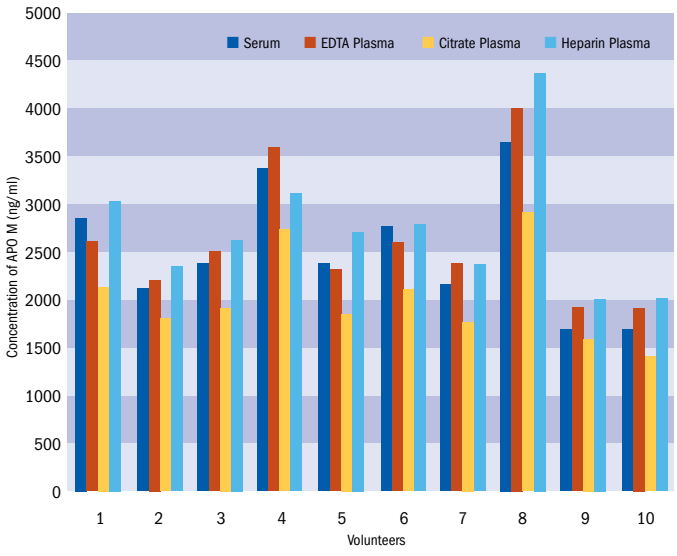
Linearity

Serum samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	5207.4	-	-
	2x	2697.6	2603.7	103.6
	4x	1437. 8	1301.8	110.4
	8x	721.7	650.9	110.9
2	-	4437.5	-	-
	2x	2224.5	2218.8	103.3
	4x	1167.9	1109.4	105.3
	8x	639.2	554.7	115.2

Effect of sample matrix

EDTA, citrate and heparin plasma samples were compared to respective serum samples from the same 10 individuals. Results are shown below:



Summary of protocol

- Reconstitute Master Standard and prepare set of Standards
- Dilute samples (5x)
- Add 100 µl Standards and samples
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Biotin Labelled Antibody
- Incubate at RT for 1 hour/300 rpm
- Wash plate 3 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min/300 rpm
- Wash plate 3 times
- Add 100 µl Substrate Solution
- Incubate at RT for 10 min
- Add 100 µl Stop Solution
- Read absorbance and calculate results

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Cross-reactivity

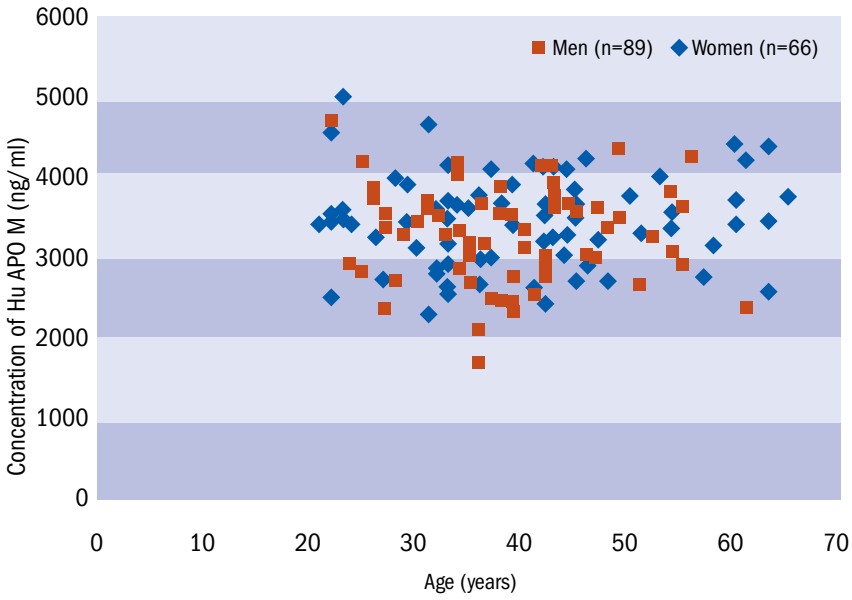
Mammalian serum Sample	Bovine	Cat	Dog	Goat	Hamster	Horse	Monkey	Mouse	Pig	Rabbit	Rat	Sheep
Observed cross-reactivity	no	no	no	no	no	no	no	no	no	no	no	no

Preliminary Population Data

The following results were obtained when serum samples from 165 unselected donors (90 men + 75 women) 20 - 69 years old were assayed with the BioVendor Human Apolipoprotein M ELISA in our laboratory:

Age and Sex Dependent Distribution of APO M

Sex	Age (years)	n	Mean APO M (ng/ml)	Median APO M (ng/ml)	SD APO M (ng/ml)	Min. APO M (ng/ml)	Max. APO M (ng/ml)
Male	20-39	44	3450.26	3479.32	584.99	2280.22	5012.96
	40-69	45	3553.43	3600.27	580.41	2435.98	5522.86
Female	20-39	38	3225.54	3292.15	496.69	1678.85	4694.47
	40-69	28	3369.56	3381.81	637.03	2355.14	4357.62



Related products

- RD172129100 Apolipoprotein M Human HEK293
- RD191236100R Apolipoprotein H/Beta2-GP1 Human ELISA
- RD193118200R Apolipoprotein D Human ELISA
- RD194034200R Clusterin Human ELISA

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