QUANTITATIVE DETERMINATION OF HUMAN PCSK9 (PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9) ELISA

NEW PRODUCT

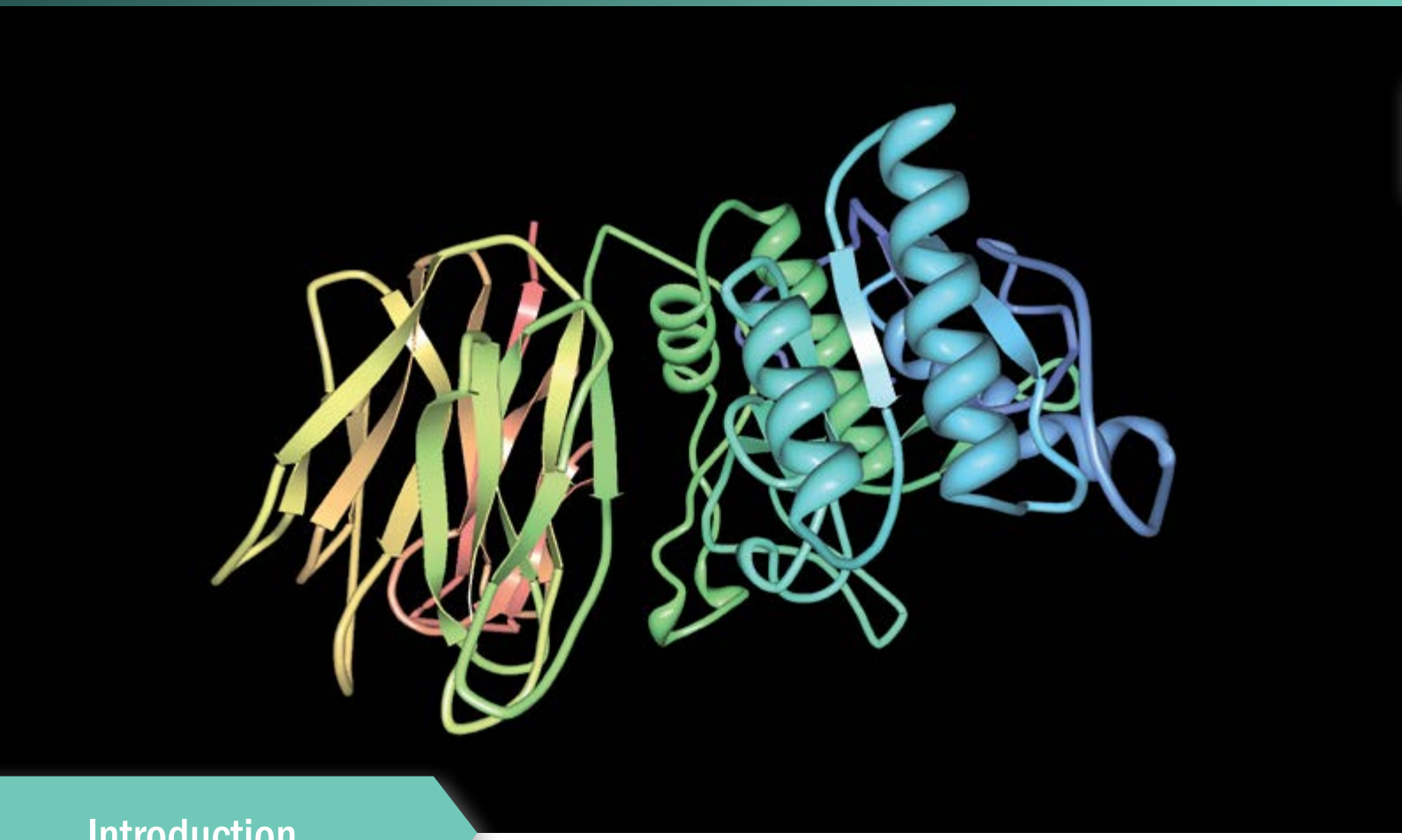
Human PCSK9 (Proprotein convertase subtilisin/kexin type 9) ELISA

- High sensitivity (9 pg/ml)
- Excellent analytical characteristics
- Validated for human serum and plasma samples (EDTA, citrate, heparin)

CARDIOVASCULAR DISEASE
DIABETOLOGY
LIPOPROTEIN METABOLISM

BioVendor
Research
and Diagnostic Products

HUMAN PCSK9 (PROPROTEIN CONVERTASE SUBTILISIN/KEXIN TYPE 9) ELISA



Introduction

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that plays an important role in the regulation of serum low-density lipoprotein (LDL) cholesterol by downregulation of LDL receptor, and as such is considered a novel target in cholesterol lowering therapy. LDL cholesterol (LDL-C) binds to LDL receptors (LDLRs) on the surface of hepatic cell where the complex is internalized and transported to the endosome. LDL-C dissociates from the receptor and is catabolized whereas the LDLR is recycled to the cell surface for continued clearance of serum cholesterol. PCSK9 affects the receptor recycling pathway by binding to the LDLR and causing degradation of the receptor within the endosome/lysosome compartment. Degradation of the LDLR results in decreased clearance of serum cholesterol, and as a result a higher risk of hypercholesterolemia.

Human genetic studies have shown that "gain-of-function" (GOF) mutations in the PCSK9 gene can lead to a form of familial hypercholesterolemia with a higher risk of cardiovascular disease. In contrast, humans with "loss-of-function" (LOF) mutations in the PCSK9 gene have lower serum cholesterol levels and a lower incidence of cardiovascular disease. Thus PCSK9 had a key impact not only on circulating LDL-C level but also on cardiovascular risk and atherosclerotic process.

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BioVendor Human PCSK9 ELISA (RD191473200R)

Intended use

The RD191473200R Human PCSK9 ELISA is a sandwich enzyme immunoassay for the quantitative measurement of Proprotein convertase subtilisin/kexin type 9 (PCSK9).

- The total assay time is less than 3.5 hours
- The kit measures PCSK9 protein in human serum and plasma samples (EDTA, citrate, heparin)
- Assay format is 96 wells
- Standard is recombinant protein
- Components of the kit are provided ready to use, concentrated or lyophilized

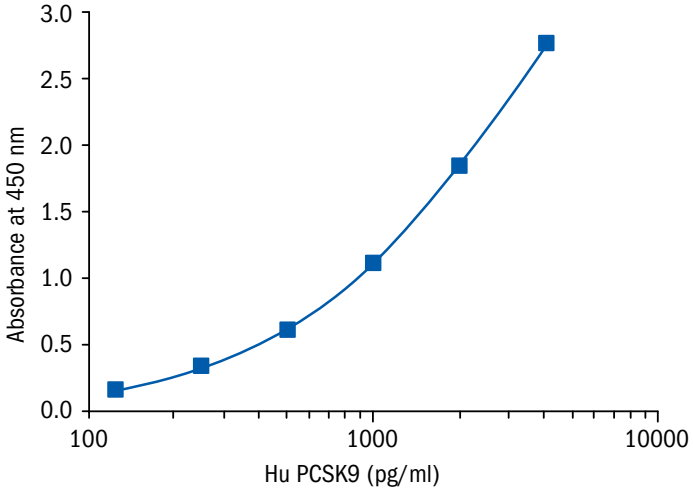
Clinical application

- Cardiovascular disease
- Diabetology
- Lipoprotein metabolism

Test principle

In the BioVendor Human PCSK9 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-PCSK9 antibody. After 60 minutes incubation and washing, biotin labelled polyclonal anti-PCSK9 antibody is added and incubated for 60 minutes with captured PCSK9. After another washing, streptavidin-HRP conjugate is added. After 30 minutes 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 PCSK9. A standard curve is constructed by plotting absorbance values against concentrations of standards, and concentrations of unknown samples are determined using this standard curve.

HUMAN PCSK9 ELISA CAT. NO.: RD191473200R	
Assay format	Sandwich ELISA, Biotin-labelled antibody, 96 wells/kit
Samples	Serum, plasma
Standards	125 – 4 000 pg/ml
Limit of detection	9 pg/ml



Precision

Inter-assay (Run-to-Run) (n=6)

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
1	269	14.1	5.2
2	479	25.4	5.3

Spiking recovery

Samples were spiked with different amounts of human PCSK9 and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	128.4	-	-
	928.5	878.4	105.7
	521.1	503.4	103.5
	372.6	315.9	103.7
2	120.3	-	-
	849.9	870.3	97.7
	462.9	495.3	93.5
	312.9	307.8	101.7

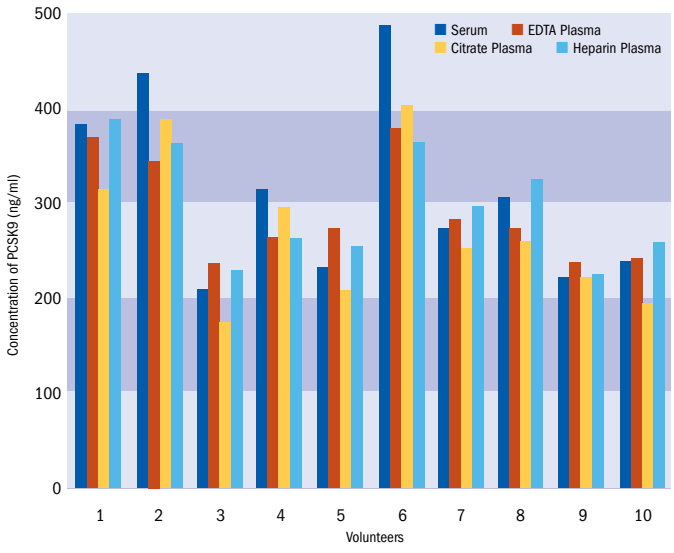
Linearity

Samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
1	-	194.1	-	-
	2x	104.4	97.1	107.6
	4x	49.8	48.5	102.6
	8x	25.9	24.3	106.7
2	-	212.8	-	-
	2x	97.7	106.4	91.8
	4x	46.3	53.2	87.0
	8x	23.3	26.6	87.6

Effect of sample matrix

EDTA, citrate and heparin plasmas 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
- Add 100 µl Standards and samples
- Incubate at RT for 1 hours with shaking 300 rpm
- Wash plate 3 times
- Prepare Biotin Labelled Antibody Solution
- Add 100 µl Biotin Labelled Antibody
- Incubate at RT for 1 hour with shaking 300 rpm
- Wash plate 3 times
- Add 100 µl Streptavidin-HRP Conjugate
- Incubate at RT for 30 min with shaking 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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Related products

· RD172473100 Proprotein Convertase Subtilisin/Kexin Type 9 Human HEK293

References

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