BioVendor Group

ENG

Instructions for Use:

HUMAN PCSK9 ELISA

Catalogue number:

RD191473200R

European Union:

IVD CE

Rest of the world:

For research use only!

BioVendor R&D

BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.007.A	ENG.008.A
”History of changes” added.	
Chapter 9.: A sentence “Centrifuge liquid containing microtube vials before opening“ added.	
Chapter 19. added	

1. INTENDED USE

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

Features

- **European Union: for *in vitro* diagnostic use**
- **Rest of the world: for research use only!**
- The total assay time is less than 3.5 hours
- Serum and plasma (EDTA, citrate, heparin)
- Assay format is 96 wells
- Quality Controls are human serum based
- Standard is recombinant protein based
- Components of the kit are provided ready to use, concentrated or lyophilized

2. STORAGE, EXPIRATION

Store the complete kit at 2-8°C. Under these conditions, the kit is stable until the expiration date (see label on the box).

For stability of opened reagents see Chapter 9.

3. INTRODUCTION

Proprotein convertase subtilisin/kexin type 9 (PCSK9) was first identified in 2003 and named as neural apoptosis regulated convertase 1 (NARC-1) [1]. It is the ninth member of the proprotein convertase family which includes proteases that process protein and peptide precursors trafficking through regulated or constitutive branches of the secretory pathway.
PCSK9 is a serine protease mainly expressed in the liver and less in intestine and kidney [3].
PCSK9 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 [5]. 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 [6,7]. PCSK9 affects the receptor recycling pathway by binding to the LDLR and causing degradation of the receptor within the endosome/lysosome compartment [6,8,9]. Degradation of the LDLR results in decreased clearance of serum cholesterol, and as a result a higher risk of hypercholesterolemia [10] .
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 [7,8,1,11]. Thus PCSK9 had a key impact not only on circulating LDL-C level but also on cardiovascular risk and atherosclerotic process [12].

Areas of investigation:
Cardiovascular diseases
Diabetology
Lipid metabolism

4. TEST PRINCIPLE

In the BioVendor Human PCSK9 ELISA, standards and samples are incubated in microplate wells pre-coated with polyclonal anti-human PCSK9 antibody. After 60 minutes incubation followed by washing, biotin labelled polyclonal anti-human PCSK9 antibody is added and incubated with captured PCSK9 for 60 minutes. 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.

5. PRECAUTIONS

- **For professional use only**
- Wear gloves and laboratory coats when handling immunodiagnostic materials
- Do not drink, eat or smoke in the areas where immunodiagnostic materials are being handled
- This kit contains components of human origin. These materials were found non-reactive for HBsAg, HCV antibody and for HIV 1/2 antigen and antibody. However, these materials should be handled as potentially infectious, as no test can guarantee the complete absence of infectious agents
- This kit contains components of animal origin. These materials should be handled as potentially infectious
- Avoid contact with the acidic Stop Solution and Substrate Solution, which contains hydrogen peroxide and tetramethylbenzidine (TMB). Wear gloves and eye and clothing protection when handling these reagents. Stop and/or Substrate Solutions may cause skin/eyes irritation. In case of contact with the Stop Solution and the Substrate Solution wash skin/eyes thoroughly with water and seek medical attention, when necessary
- The materials must not be pipetted by mouth

6. TECHNICAL HINTS

- Reagents with different lot numbers should not be mixed
- Use thoroughly clean glassware
- Use deionized (distilled) water, stored in clean containers
- Avoid any contamination among samples and reagents. For this purpose, disposable tips should be used for each sample and reagent
- Substrate Solution should remain colourless until added to the plate. Keep Substrate Solution protected from light
- Stop Solution should remain colourless until added to the plate. The colour developed in the wells will turn from blue to yellow immediately after the addition of the Stop Solution. Wells that are green in colour indicate that the Stop Solution has not been mixed thoroughly with the Substrate Solution
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements

7. REAGENT SUPPLIED

Kit Components	State	Quantity
Antibody Coated Microtiter Strips	ready to use	96 wells
Biotin Labelled Antibody Conc. (100x)	concentrated	0.13 ml
Streptavidin-HRP Conjugate	ready to use	13 ml
Master Standard	lyophilized	2 vials
Quality Control HIGH	lyophilized	2 vials
Quality Control LOW	lyophilized	2 vials
Dilution Buffer	ready to use	75 ml
Wash Solution Conc. (10x)	concentrated	100 ml
Substrate Solution	ready to use	13 ml
Stop Solution	ready to use	13 ml

8. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized (distilled) water
- Test tubes for diluting samples
- Glassware (graduated cylinder and bottle) for Wash Solution (Dilution Buffer)
- Precise pipettes to deliver 10-1000 µl with disposable tips
- Multichannel pipette to deliver 100 µl with disposable tips
- Absorbent material (e.g. paper towels) for blotting the microtitrate plate after washing
- Vortex mixer
- Orbital microplate shaker capable of shaking at approximately 300 rpm
- Microplate washer (optional). [Manual washing is possible but not preferable.]
- Microplate reader with 450 ± 10 nm filter, preferably with reference wavelength 630 nm (alternatively another one from the interval 550-650 nm)
- Software package facilitating data generation and analysis (optional)

9. PREPARATION OF REAGENTS

All reagents need to be brought to room temperature prior to use.

Centrifuge liquid containing microtube vials before opening.

Always prepare only the appropriate quantity of reagents for your test.

Do not use components after the expiration date marked on their label.

Assay reagents supplied ready to use:

Antibody Coated Microtiter Strips

Stability and storage:
Return the unused strips to the provided aluminium zip-sealed bag with desiccant and seal carefully. Remaining Microtiter Strips are stable 3 months when stored at 2-8°C and protected from the moisture.

Streptavidin-HRP Conjugate

Dilution Buffer

Substrate Solution

Stop Solution

Stability and storage:
Opened reagents are stable 3 months when stored at 2-8°C.

Assay reagents supplied concentrated or lyophilized:

PCSK9 Master Standard

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution of standard!!!
Reconstitute the lyophilized Master Standard with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
The resulting concentration of PCSK9 in the stock solution is **4 000 pg/ml**.

Prepare set of standards using Dilution Buffer as follows:

Volume of Standard	Dilution Buffer	Concentration
Stock	-	4 000 pg/ml
250 µl of stock	250 µl	2 000 pg/ml
250 µl of 2 000 pg/ml	250 µl	1 000 pg/ml
250 µl of 1 000 pg/ml	250 µl	500 pg/ml
250 µl of 500 pg/ml	250 µl	250 pg/ml
250 µl of 250 pg/ml	250 µl	125 pg/ml

Prepared Standards are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Master Standard and/or diluted standard solutions.

Quality Controls HIGH, LOW

Refer to the Certificate of Analysis for current volume of Dilution Buffer needed for reconstitution and for current Quality Control concentration!!!
Reconstitute each Quality Control (HIGH and LOW) with Dilution Buffer just prior to the assay. Let it dissolve at least 15 minutes with occasional gentle shaking (not to foam).
Reconstituted Quality Controls are ready to use, do not dilute them.

Stability and storage:
Do not store the reconstituted Quality Controls.

Note:
Concentration of analyte in Quality Controls need not be anyhow associated with normal and/or pathological concentrations in serum or another body fluid. Quality Controls serve just for control that the kit works in accordance with IFU and CoA and that ELISA test was carried out properly.

Biotin Labelled Antibody Conc. (100x)

Prepare the working Biotin Labelled Antibody solution by adding 1 part Biotin Labelled Antibody Concentrate (100x) to 99 parts Dilution Buffer.
Example: 10 µl of Biotin Labelled Antibody Concentrate (100x) + 990 µl of Dilution Buffer for 1 strip (8 wells). **Mix well** (not to foam).

Stability and storage:
Opened Biotin Labelled Antibody Concentrate (100x) is stable 3 months when stored at 2–8°C.
Do not store the diluted Biotin Labelled Antibody solution.

Wash Solution Conc. (10x)

Dilute Wash Solution Concentrate (10x) ten-fold in distilled water to prepare a 1x working solution.
Example: 100 ml of Wash Solution Concentrate (10x) + 900 ml of distilled water for use of all 96-wells.

Stability and storage:
The diluted Wash Solution is stable 1 month when stored at 2-8°C. Opened Wash Solution Concentrate (10x) is stable 3 months when stored at 2-8°C.

10. PREPARATION OF SAMPLES

The kit measures PCSK9 in serum and plasma samples (EDTA, citrate, heparin).

Samples should be assayed immediately after collection or should be stored frozen. Thoroughly mix thawed samples just prior to the assay and avoid repeated freeze/thaw cycles, which may cause erroneous results. Avoid using hemolyzed or lipemic serum samples.

An appropriate dilution should be assessed by the researcher prior to batch measurement.

Recommended starting dilution 200x.

Dilute serum or plasma samples 200x with Dilution Buffer just prior to the assay in two steps as follow:

Dilution A (20x):
Add 10 µl of sample into 190 µl of Dilution Buffer. **Mix well** (not to foam). Vortex is recommended.

Dilution B (10x):
Add 15 µl of Dilution A into 135 µl of Dilution Buffer for singlets, or preferably 30 µl of Dilution A + 270 µl of Dilution Buffer for duplicates, to prepare final dilution **(200x)**.
Mix well (not to foam). Vortex is recommended.

Stability and storage:
Samples should be stored at -20°, or preferably at -70°C for long-term storage. Avoid repeated freeze/ thaw cycles.
Do not store the diluted samples.

Note: It is recommended to use a precise pipette and a careful technique to perform the dilution in order to get precise results.

11. ASSAY PROCEDURE

1. Pipet **100 µl** of Standards, Dilution Buffer (=Blank) and diluted samples, preferably in duplicates, into the appropriate wells. See *Figure 1* for example of work sheet.
2. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
3. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
4. Pipet **100 µl** of Biotin Labelled Antibody solution into each well.
5. Incubate the plate at room temperature (ca. 25°C) for **1 hour**, shaking at ca. 300 rpm on an orbital microplate shaker.
6. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
7. Pipet **100 µl** of Streptavidin-HRP Conjugate into each well.
8. Incubate the plate at room temperature (ca. 25°C) for **30 minutes**, shaking at ca. 300 rpm on an orbital microplate shaker.
9. Wash the wells 5-times with Wash Solution (0.35 ml per well). After final wash, invert and tap the plate strongly against paper towel.
10. Add **100 µl** of Substrate Solution. (Avoid exposing the microtiter plate to direct sunlight. Covering the plate with e.g. aluminium foil is recommended.)
11. Incubate the plate for **15 minutes** at room temperature. (The incubation time may be extended [up to 20 minutes] if the reaction temperature is below than 20°C). No shaking!
12. Stop the colour development by adding **100 µl** of Stop Solution.
13. Determine the absorbance of each well using a microplate reader set to 450 nm, preferably with the reference wavelength set to 630 nm (acceptable range: 550-650 nm). Subtract readings at 630 nm (550-650 nm) from the readings at 450 nm. **The absorbance should be read within 5 minutes following step 12.**

Note 1: If some samples and standard/s have absorbances above the upper limit of your microplate reader, perform a second reading at 405 nm. A new standard curve, constructed using the values measured at 405 nm, is used to determine PCSK9 concentration of off-scale standards and samples. The readings at 405 nm should not replace the readings for samples that were “in range” at 450 nm.

Note 2: Manual washing: Aspirate wells and pipet 0.35 ml Wash Solution into each well. Aspirate wells and repeat four times. After final wash, invert and tap the plate strongly against paper towel. Make certain that Wash Solution has been removed entirely.

	strip 1+2	strip 3+4	strip 5+6	strip 7+8	strip 9+10	strip 11+12
A	**Standard 4 000**	**QC LOW**	Sample 8	Sample 16	Sample 24	Sample 32
B	**Standard 2 000**	Sample 1	Sample 9	Sample 17	Sample 25	Sample 33
C	**Standard 1 000**	Sample 2	Sample 10	Sample 18	Sample 26	Sample 34
D	**Standard 500**	Sample 3	Sample 11	Sample 19	Sample 27	Sample 35
E	**Standard 250**	Sample 4	Sample 12	Sample 20	Sample 28	Sample 36
F	**Standard 125**	Sample 5	Sample 13	Sample 21	Sample 29	Sample 37
G	**Blank**	Sample 6	Sample 14	Sample 22	Sample 30	Sample 38
H	**QC HIGH**	Sample 7	Sample 15	Sample 23	Sample 31	Sample 39

Figure 1: Example of a work sheet.

12. CALCULATIONS

Most microplate readers perform automatic calculations of analyte concentration. The Standard curve is constructed by plotting the mean absorbance (Y) of Standards against the known concentration (X) of Standards in logarithmic scale, using the four-parameter algorithm. Results are reported as concentration of PCSK9 (pg/ml) in samples.

Alternatively, the logit log function can be used to linearize the standard curve (i.e. logit of the mean absorbance (Y) is plotted against log of the known concentration (X) of standards).

The measured concentration of samples calculated from the standard curve must be multiplied by their respective dilution factor, because samples have been diluted prior to the assay, e.g. 500 pg/ml (from standard curve) x 200 (dilution factor) = 100 000 pg/ml = 100 ng/ml.

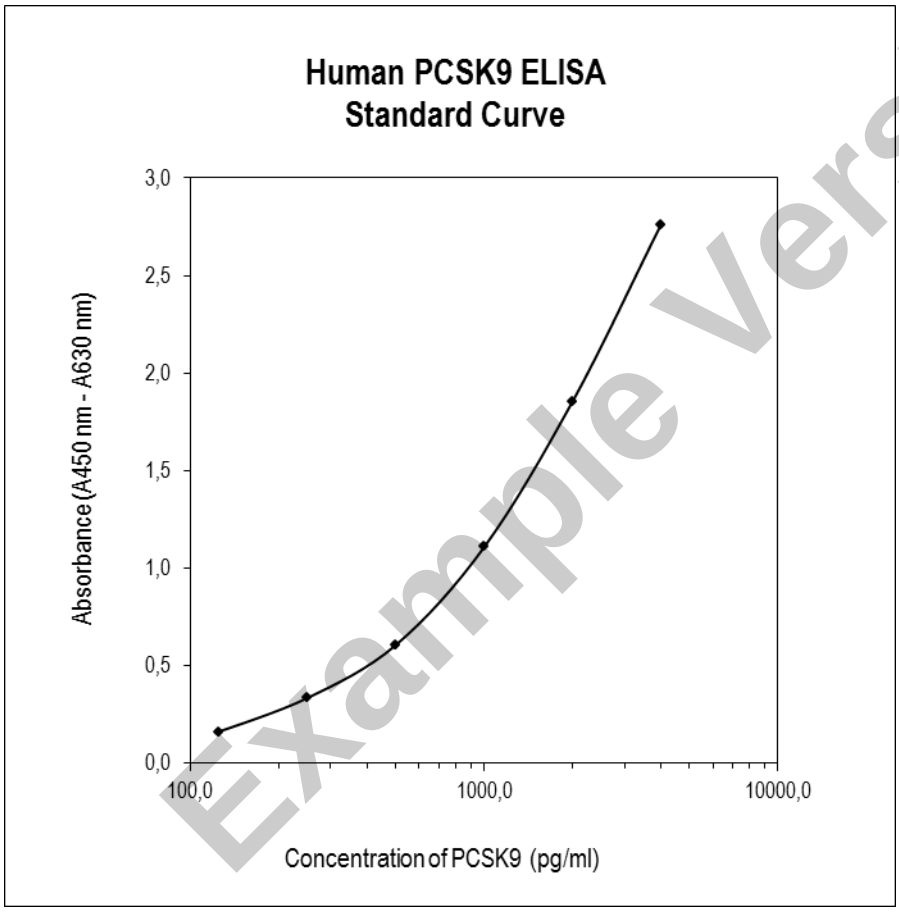


Figure 2: Typical Standard Curve for Human PCSK9 ELISA.

13. PERFORMANCE CHARACTERISTICS

Typical analytical data of BioVendor Human PCSK9 ELISA are presented in this chapter.

Sensitivity

Limit of Detection (LOD) (defined as concentration of analyte giving absorbance higher than mean absorbance of blank* plus three standard deviations of the absorbance of blank: $A_{blank} + 3xSD_{blank}$) is calculated from the real PCSK9 values in wells and is 9 pg/ml.
*Dilution Buffer is pipetted into blank wells.

Limit of assay

Samples with absorbances exceeding the absorbance of the highest standard should be measured again with higher dilution. The final concentration of samples calculated from the standard curve must be multiplied by the respective dilution factor.

Specificity

The antibodies used in this ELISA are specific for human PCSK9.
We observed no interference of hemoglobin (1.0 mg/ml), bilirubin (170 µmol/l) and triglycerides (5.0 mmol/l).

Sera of several mammalian species were measured in the assay. See results below.
For details please contact us at info@biovendor.com.

Mammalian serum sample	Observed crossreactivity
Bovine	no
Cat	no
Dog	no
Goat	no
Hamster	no
Horse	no
Monkey	yes
Mouse	yes
Pig	yes
Rabbit	no
Rat	no
Sheep	no

Presented results are multiplied by respective dilution factor.

Precision

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Serum 1	479.2	25.4	5.3
Serum 2	269.0	14.1	5.2

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

Sample	Mean (ng/ml)	SD (ng/ml)	CV (%)
Serum 1	969.0	38.7	4.0
Serum 2	874.7	65.8	7.5

Spiking Recovery

Samples were spiked with different amounts of PCSK9 and assayed.

Sample	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	204.0	-	-
	311.4	279.0	111.6
	380.4	354.0	107.5
	516.4	504.0	102.5
Serum 2	174.4	-	-
	251.4	249.4	100.8
	303.3	324.4	93.5
	441.4	474.4	93.0

Linearity

Samples were serially diluted with Dilution Buffer and assayed.

Sample	Dilution	Observed (ng/ml)	Expected (ng/ml)	Recovery O/E (%)
Serum 1	-	607.7	-	-
	2x	310.7	303.9	102.3
	4x	137.7	151.9	90.6
	8x	73.5	76.0	96.8
Serum 2	-	446.1	-	-
	2x	218.6	223.0	98.0
	4x	121.5	111.5	109.0
	8x	64.8	55.8	116.2

Effect of sample matrix

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

Volunteer No.	Serum (ng/ml)	Plasma (ng/ml)		
		EDTA	Citrate	Heparin
1	385.3	366.0	312.2	388.0
2	433.1	339.8	388.2	361.3
3	209.0	231.9	173.4	227.2
4	313.2	260.5	295.3	261.6
5	232.4	274.7	202.0	255.7
6	488.6	371.8	405.4	360.7
7	272.3	280.7	252.3	297.2
8	301.8	272.9	257.6	325.6
9	291.5	235.3	217.5	225.1
10	236.8	242.2	194.4	254.9
Mean (pg/ml)	**309.2**	**287.6**	**269.9**	**295.7**
Mean Plasma/Serum (%)	**-**	**93.0**	**87.3**	**95.6**
Coefficient of Determination R^2	**-**	**0.85**	**0.96**	**0.78**

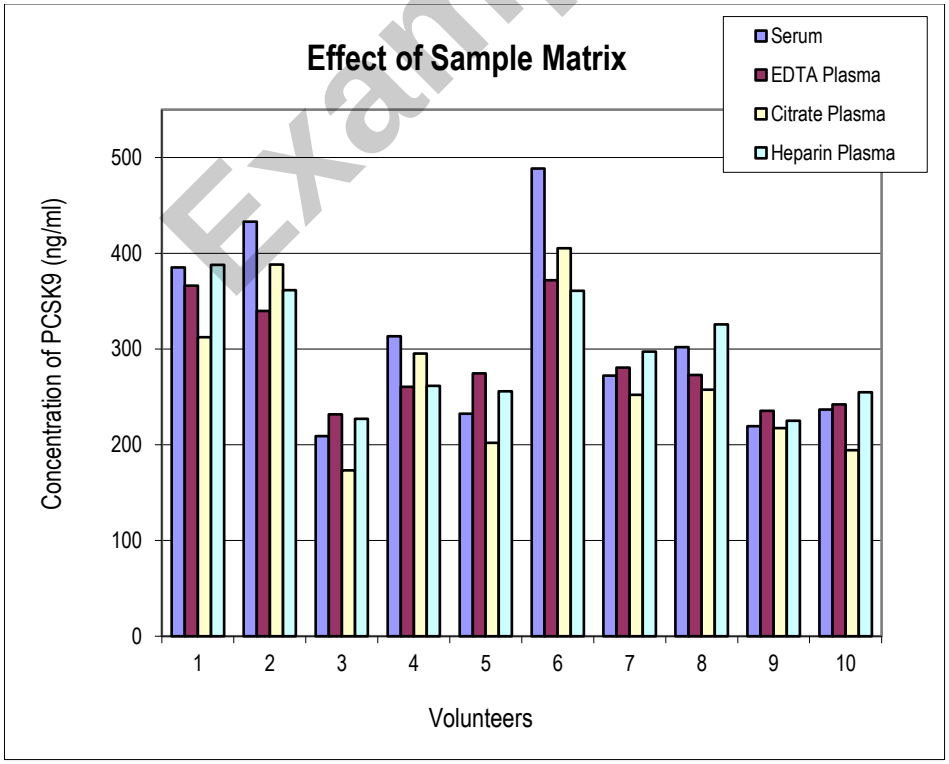


Figure 3: PCSK9 levels measured using Human PCSK9 ELISA in serum, EDTA, citrate, and heparin plasma, respectively, from the same 10 individuals.

Stability of samples stored at 2-8°C

Samples should be stored at –20°C. However, no decline in concentration of PCSK9 was observed in serum and plasma samples after 7 days when stored at 2-8°C. To avoid microbial contamination, samples were treated with ε-aminocaproic acid and sodium azide, resulting in the final concentration of 0.03% and 0.1%, respectively.

Sample	Incubation Temp., Period	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	-20°C	295.0	412.9	315.9	368.2
	2-8°C, 1 day	261.1	416.0	345.9	367.6
	2-8°C, 7 days	251.9	349.6	312.8	342.4
2	-20°C	342.8	400.9	358.2	432.5
	2-8°C, 1 day	334.8	406.2	348.1	406.0
	2-8°C, 7 days	300.1	416.4	375.0	357.8
3	-20°C	466.9	488.7	442.2	522.4
	2-8°C, 1 day	497.1	421.8	426.2	525.0
	2-8°C, 7 days	373.7	374.4	346.8	414.7

Effect of Freezing/Thawing

No decline was observed in concentration of human PCSK9 in serum and plasma samples after repeated (5x) freeze/thaw cycles. However it is recommended to avoid unnecessary repeated freezing/thawing of the samples.

Sample	Number of f/t cycles	Serum (ng/ml)	Plasma (ng/ml)		
			EDTA	Citrate	Heparin
1	1x	209.6	252.4	208.0	263.4
	3x	235.1	267.9	209.0	222.6
	5x	187.5	235.1	201.4	242.9
2	1x	222.2	272.8	207.9	235.9
	3x	202.1	272.8	243.7	257.0
	5x	212.9	277.2	211.0	208.1
3	1x	146.9	181.2	165.8	186.0
	3x	176.1	198.0	165.2	186.3
	5x	145.1	180.0	157.4	172.4

14. DEFINITION OF THE STANDARD

Recombinant Human Proprotein Convertase Subtilisin/Kexin Type 9, expressed in HEK293, is used as the standard. The protein has 671 amino acids and molecular weight 72.4 kDa.

15. PRELIMINARY POPULATION AND CLINICAL DATA

The following results were obtained when serum samples from 154 unselected donors (89 men + 65 women) 21-65 years old were assayed with the BioVendor Human PCSK9 ELISA in our laboratory.

Age dependent distribution of PCSK9

Sex	Age (years)	n	PCSK9 (ng/ml)				
			Mean	Median	SD	Min	Max
Men	21-29	17	196.9	189.4	48.2	128.4	286.8
	30-39	25	220.8	213.4	91.7	72.0	463.2
	40-49	31	231.6	224.0	77.9	108.0	450.6
	50-65	16	227.8	229.5	59.4	137.6	334.6
Women	22-29	12	147.0	150.7	38.1	89.4	205.0
	30-39	26	204.9	196.5	59.9	105.4	325.8
	40-49	19	237.7	212.4	98.3	103.4	469.0
	50-61	8	270.9	265.3	84.8	162.4	380.4

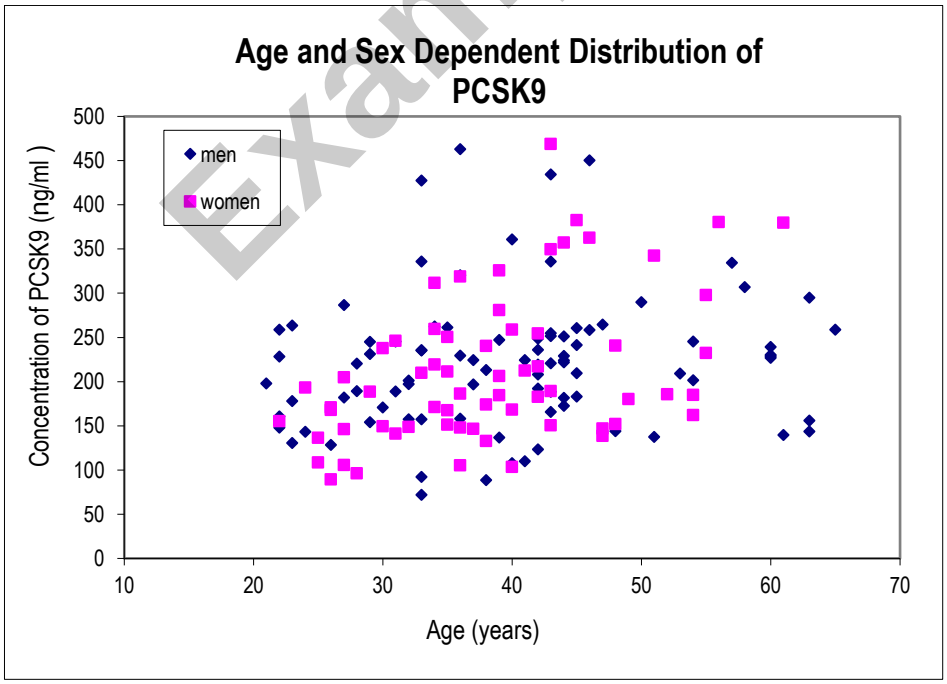


Figure 4: Human PCSK9 concentration plotted against donor age and sex.

Reference range

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological references ranges for PCSK9 levels with the assay.

16. METHOD COMPARISON

The BioVendor Human PCSK9 ELISA was compared to another commercial immunoassay by measuring 12 serum samples. The following correlation graph was obtained:

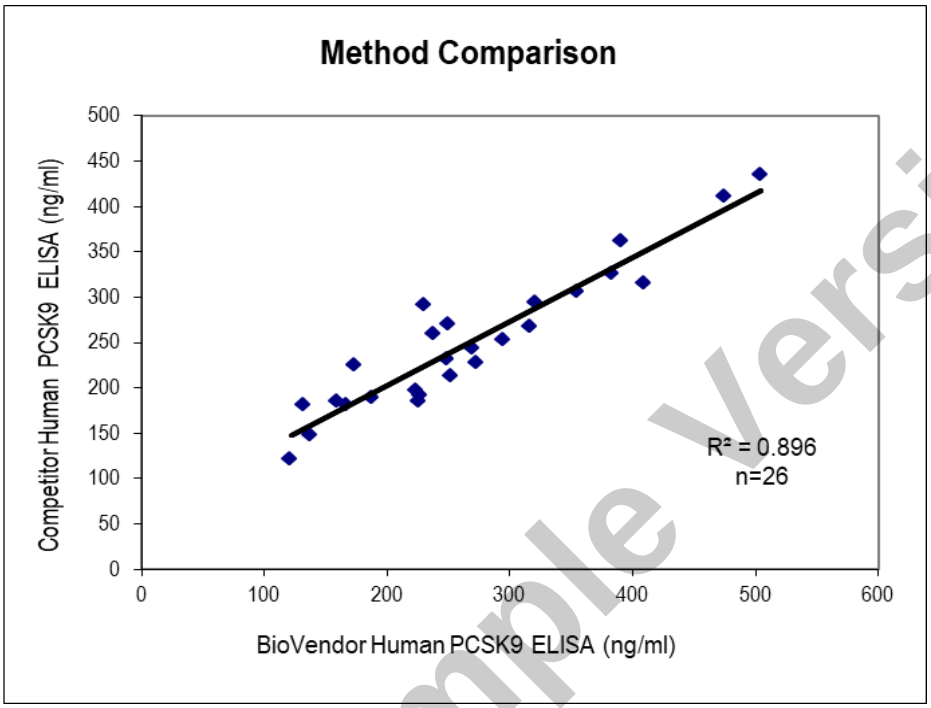


Figure 5: Method Comparison

17. TROUBLESHOOTING AND FAQS

Weak signal in all wells

Possible explanations:
- Omission of a reagent or a step
- Improper preparation or storage of a reagent
- Assay performed before reagents were allowed to come to room temperature
- Improper wavelength when reading absorbance

High signal and background in all wells

Possible explanations:
- Improper or inadequate washing
- Overdeveloping; incubation time with Substrate Solution should be decreased before addition of Stop Solution
- Incubation temperature over 30°C

High coefficient of variation (CV)

Possible explanation:
- Improper or inadequate washing
- Improper mixing Standards and samples

18. REFERENCES

References to PCSK9:

1. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M: The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. Proc Natl Acad Sci U S A. 2003;100(3):928-33
2. Seidah NG, Prat A: The proprotein convertases are potential targets in the treatment of dyslipidemia. J Mol Med (Berl). 2007;85(7):685-96.
3. Seidah NG, Prat A: The biology and therapeutic targeting of the proprotein convertases. Nat Rev Drug Discov 2012;11(5):367-83
4. Seidah NG, Awan Z, Chrétien M, Mbikay M: PCSK9: a key modulator of cardiovascular health. Circ Res. 2014;114(6):1022-36
5. Colbert A, Umble-Romero A, Prokop S, Xu R, Gibbs J, Pederson S: Characterization of a quantitative method to measure free proprotein convertase subtilisin/kexin type 9 in human serum. MAb 2014;6(4):1103-13
6. Konrad RJ, Troutt JS, Cao G: Effects of currently prescribed LDL-C-lowering drugs on PCSK9 and implications for the next generation of LDL-C-lowering agents. Lipids Health Dis 2011;10:38
7. Chan JC, Piper DE, Cao Q, Liu D, King C, Wang W, Tang J, Liu Q, Higbee J, Xia Z, Di Y, Shetterly S, Arimura Z, Salomonis H, Romanow WG, Thibault ST, Zhang R, Cao P, Yang XP, Yu T, Lu M, Retter MW, Kwon G, Henne K, Pan O, Tsai MM, Fuchslocher B, Yang E, Zhou L, Lee KJ, Daris M, Sheng J, Wang Y, Shen WD, Yeh WC, Emery M, Walker NP, Shan B, Schwarz M, Jackson SM: A proprotein convertase subtilisin/kexin type 9 neutralizing

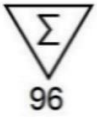
antibody reduces serum cholesterol in mice and nonhuman primates. Proc Natl Acad Sci 2009;106(24):9820-5
8. Horton JD, Cohen JC, Hobbs HH: PCSK9: a convertase that coordinates LDL catabolism. J Lipid Res 2009;50 Suppl:S172-7
9. Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ: High-dose atorvastatin causes a rapid sustained increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. J Lipid Res. 2010;51(9):2714-21
10. Alborn WE, Cao G, Careskey HE, Qian YW, Subramaniam DR, Davies J, Conner EM, Konrad RJ: Serum proprotein convertase subtilisin kexin type 9 is correlated directly with serum LDL cholesterol. Clin Chem. 2007;53(10):1814-9
11. Careskey HE, Davis RA, Alborn WE, Troutt JS, Cao G, Konrad RJ: Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. J Lipid Res 2008 Feb;49(2):394-8
12. Guo YL, Zhang W, Li JJ: PCSK9 and lipid lowering drugs. Clin Chim Acta. 2014;437:66-71
13. Raal F, Panz V, Immelman A, Pilcher G: Elevated PCSK9 levels in untreated patients with heterozygous or homozygous familial hypercholesterolemia and the response to high-dose statin therapy. J Am Heart Assoc 2013;2(2)
14. Almontashiri NA, Vilmundarson RO, Ghasemzadeh N, Dandona S, Roberts R, Quyyumi AA, Chen HH, Stewart AF: Plasma PCSK9 levels are elevated with acute myocardial infarction in two independent retrospective angiographic studies. PLoS One 2014;9(9)
15. Leander K, Mälarstig A, Van't Hooft FM, Hyde C, Hellénius ml, Troutt JS, Konrad RJ, Öhrvik J, Hamsten A, de Faire 2: Circulating Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) Predicts Future Risk of Cardiovascular Events Independently of Established Risk Factors. Circulation 2016;133(13):1230-9
16. Chao TH, Chen IC, Li YH, Lee PT, Tseng SY: Plasma Levels of Proprotein Convertase Subtilisin/Kexin Type 9 Are Elevated in Patients With Peripheral Artery Disease and Associated With Metabolic Disorders and Dysfunction in Circulating Progenitor Cells. J Am Heart Assoc. 2016;5(5)

For more references on this product see our web pages at www.biovendor.com.

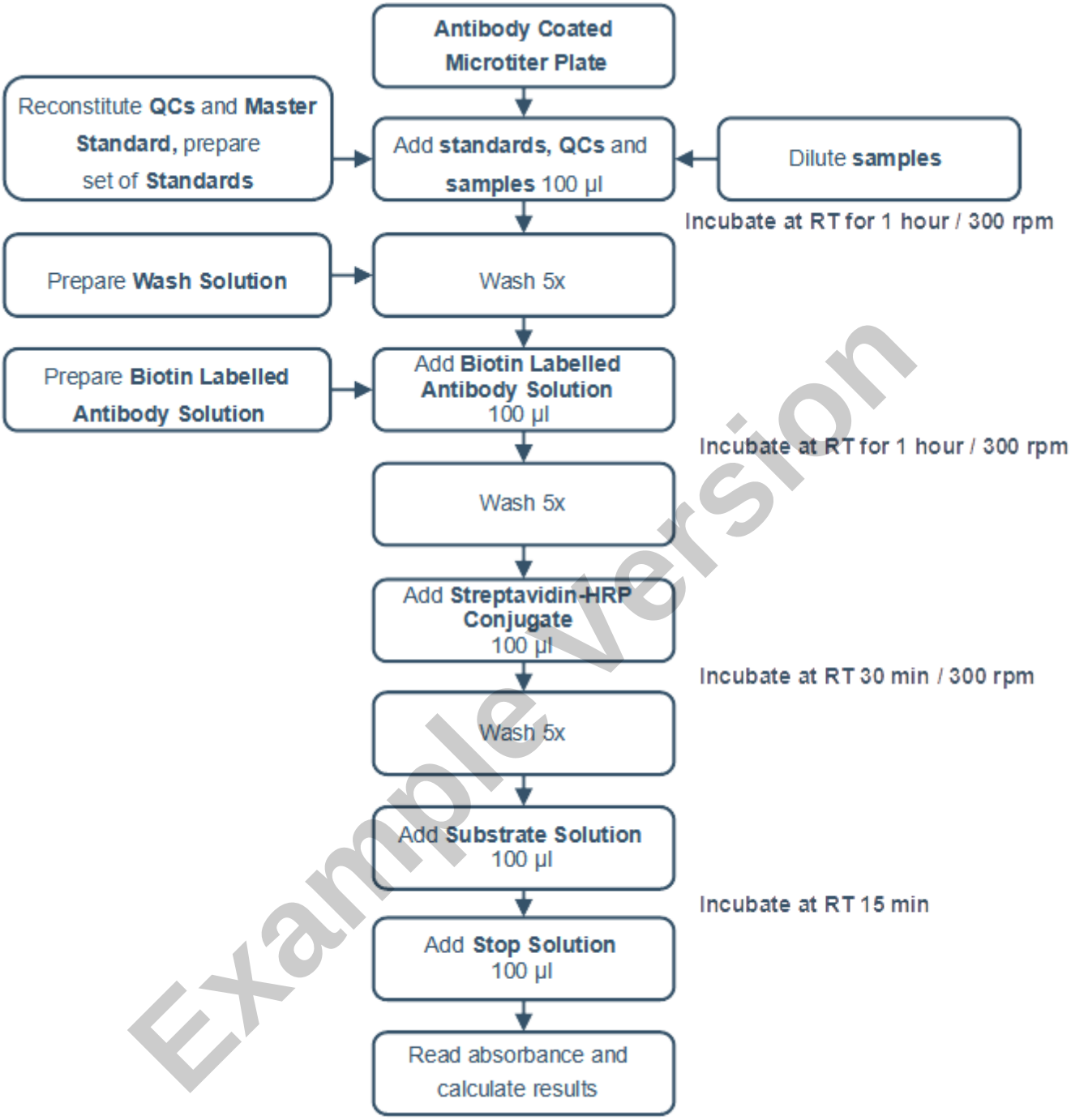
19. ADDITIONAL INFORMATION

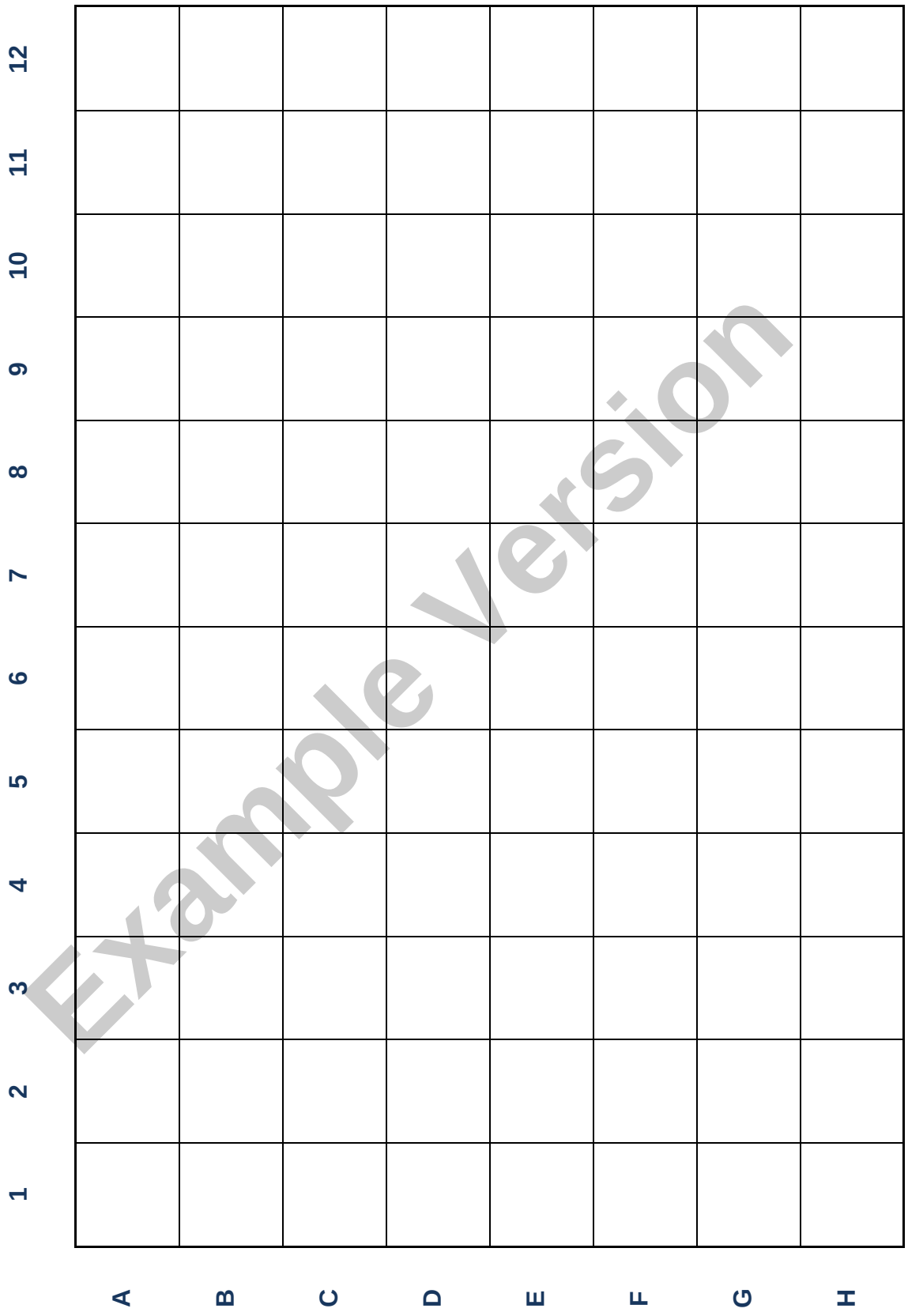
Any serious incident occurring in connection with the device must be reported to the manufacturer and to the competent authority of the Member State in which the user or patient is located.

20. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks
	In vitro diagnostic medical device
	CE marking of conformity

21. ASSAY PROCEDURE - SUMMARY





BioVendor – Laboratorní medicína a.s.
Karásek 1767/1, 621 00 Brno, Czech Republic
+420 549 124 185
info@biovendor.com
sales@biovendor.com
www.biovendor.com

Date of last revision: 12.04.2023