HUMAN PROPROTIN CONVERTASE 9 (PCSK9) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN PCSK9 CONCENTRATIONS IN SERUM, PLASMA AND CELL CULTURE SUERNATES.

PURCHASE INFORMATION:

ELISA NAME	HUMAN PCSK9 ELISA
Catalog No.	SK00003-01
Lot No.:	
Formulation	96 T
Standard range	62.5-4000 pg/mL
Sensitivity	31 pg/mL
Sample Volume	100 µl
Dilution Factor	*100~200 (Optimal dilutions should be determined by each laboratory for each application.)*
Sample Type	Serum, EDTA plasma, Cell Culture Supernates
Specificity	Human PCSK9 only
Intra-assay Precision	4-6%
Inter-assay Precision	8-12%
Storage	4 °C

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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INTRODUCTION

Human PCSK9 immunoassay is a 3.5 - 4.5 hour solid phase ELISA designed to measure human PCSK9 in cell culture supernates, serum, and EDTA plasma. It contains recombinant human PCSK9 and antibodies raised against this protein. It has been shown to accurately quantify recombinant human PCSK9. Results obtained with naturally occurring PCSK9 samples showed linear curves that were parallel to the standard curves obtained using the kit standards. These results indicate that the immunoassay kit can be used to determine relative mass values for natural human PCSK9.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human PCSK9 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PCSK9 present is bound by the immobilized antibody. After washing away any unbound substances, a biotinylated antibody specific for PCSK9 is added to the wells. Following a wash to remove any unbound antibody-biotin reagent, HRP link Streptavidin is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of PCSK9 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

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_ The kit should not be used beyond the expiration date on the kit label.

_ Do not mix or substitute reagents with those from other lots or sources.

_ It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.

_ If samples generate values higher than the highest standard, dilute the samples with the appropriate Dilution Buffer and repeat the assay.

_ Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

_ This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
PCSK9 Microplate – 96 well microplate precoated with an anti-human PCSK9 antibody	**003-01-01**	**1 plate**
PCSK9 Standard – 4000 pg/vial of recombinant human PCSK9 in a buffered protein base with preservatives; lyophilized.	**003-01-02**	**1 vial**
PCSK9 Antibody Concentrate – 105µl/vial, 100-fold concentrated of antibody against human PCSK9 with preservatives; lyophilized.	**003-01-03**	**1 vial**
Positive Control – one vial of recombinant human PCSK9 , lyophilized (optional)	**003-01-04**	**1 vial**
Streptavidin-HRP Conjugate - 60 µl/vial, 200-fold concentrated solution of Streptavidin conjugate to HRP	**SAHRP**	**1 vial**
Dilution Buffer - 60 mL of buffered protein based solution with preservatives	**DB01**	**2 bottles**
Wash Buffer - 50 mL of 10-fold concentrated buffered surfactant, with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution - 11 mL of TMB substrate solution	**TMB01**	**1 bottle**
Stop Solution - 11 mL of 0.5M HCl	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 - 8° C for up to 12 months. For longer storage, unopened Standard, Positive Control and Antibody Concentrate should be stored at -20 or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard, Positive Control and Antibody Concentrate SHOULD BE STORED at -20 °C or -70°C for up to one month. Streptavidin-HRP Conjugate 200-fold concentrate and other components may be stored at 2 - 8°C for up to 12 months.

Microplate Wells: Return unused wells to the plastic pouch with the desiccant pack, reseal along the entire edge of zip-seal. Microplate may be stored for up to 6 months at 2 - 8° C after opening.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

PRECAUTIONS FOR USE

All reagents should be considered as potentially hazardous. The stop solution contains diluted hydrochloric acid. Appropriate care, therefore, should be taken while handling this solution.
We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20° C. Avoid repeated freeze-thaw cycles.
Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

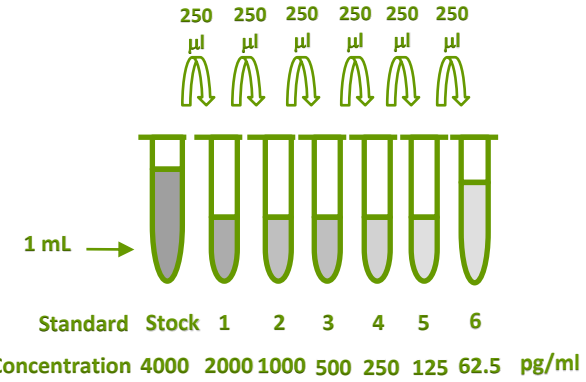
Serum and EDTA plasma samples may require a 100-200 fold dilution. A suggested 100-fold dilution is 10 µL sample + 90 µL Dilution Buffer. Following 30 µl of 10-fold diluted samples + 270 µl Dilution Buffer. A suggested 200-fold dilution is 10 µL sample + 190 µL Dilution Buffer. Following 30 µl of 20-fold diluted samples + 270 µl of Dilution Buffer.
Optimal dilutions should be determined by each laboratory for each application.
Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 50 mL of Wash Buffer Concentrate into deionized or distilled water (450 mL) to prepare 500 mL of Wash Buffer.

PCSK9 Standard - Refer to vial label for reconstitution volume. Reconstitute the **PCSK9** standard with 1.0 mL of Dilution Buffer. This reconstitution produces a stock solution of 4000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250 µL of Dilution Buffer into tubes #1 to #6. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The Dilution Buffer serves as the zero standard (0 pg/mL).

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
stock	powder	1.0 mL	4000 pg/ml
# 1	250µl of stock	250µl	2000 pg/ml
# 2	250µl of 1	250µl	1000 pg/ml
# 3	250µl of 2	250µl	500 pg/ml
# 4	250µl of 3	250µl	250 pg/ml
# 5	250µl of 4	250µl	125 pg/ml
# 6	250µl of 5	250µl	62.5 pg/ml



PCSK9 Antibody Concentrate - Reconstitute the Antibody Concentrate with 105 µl of Dilution Buffer to produce a 100-fold concentrated stock solution. Transfer it to 10.395 mL of Dilution Buffer to prepare 1x Antibody working solution.

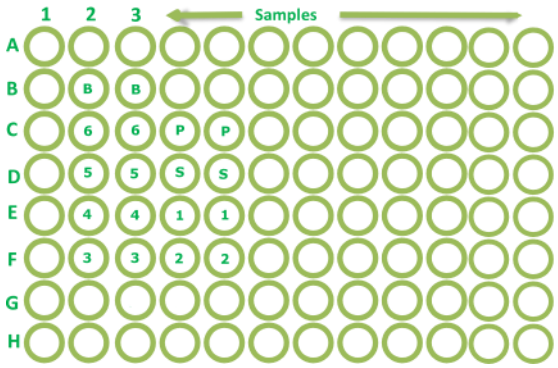
Streptavidin-HRP Conjugate - Transfer 60 µL of 200-fold concentrated stock solution to 11.94 mL of Dilution Buffer to prepare working solution. **Note:** 1x working solution of Streptavidin-HRP Conjugate should be used within a few days.

Positive Control - Reconstitute the Positive Control with 1.0 mL of Dilution Buffer. **Note:** Positive Control should be prepared and used immediately.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that blank, standards, positive control and samples be assayed in duplicates.

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess micro-plate strips from the plate frame, return them to the plastic pouch with the desiccant pack, reseal.
3. Leave well B2 and B3 as Blank. Add 100 µl per well of Dilution Buffer.
4. Add 100 µl per well of standard solution from #6 to #S (reverse order of serial dilution) to the appropriate wells (C2, C3 to F2, F3, and F4, F5 to D4, D5). Add 100 µl per well of Positive control into wells C4 and C5. Add 100 µl per well of samples into appropriate wells. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm). **Note:** Standard, Blank and PC should be assayed in duplicates.
5. Aspirate wells and wash 4 times with 300 µl of 1x Assay Wash Buffer. Blot plate on absorbent paper to remove any residual buffer.
6. Add 100 µl per well of 1x Antibody working solution. Cover or seal the plate and incubate at room temperature for 2 hours on microplate shaker (250 rpm).
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Streptavidin-HRP Conjugate working solution. Cover or seal the plate and incubate at room temperature for 60 minutes on microplate shaker. **Protect from light**.
11. Repeat the aspiration/wash as in step 5.
12. Add 100 µL of Substrate Solution to each well. Incubate for 15-25 minutes at room temperature. **Protect from light**.
13. Add 100 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.



CALCULATION OF RESULTS

Average the duplicate readings for each standard, positive control, and samples and subtract the average Blank optical density. It is recommended to use software capable of generating a log-log curve-fit. The standard curve shows relationship between

standard concentrations and corresponding O.D absorbance. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human PCSK9.

SENSITIVITY

The minimum detectable dose (MDD) of human PCSK9 was 31 pg/mL.

TYPICAL DATA

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

STANDARD (PG/ML)	AVERAGE OD450 (CORRECTED)*
Blank	0 (0.079)
62.5	0.028
125	0.043
250	0.082
500	0.182
1000	0.365
2000	0.745
4000	1.645

- **Lot No.:**
- **Positive Control: 300-600 pg/ml**

SPECIFICITY

This assay recognizes both natural and recombinant human PCSK9. The factors listed below were prepared at 2000 ng/mL in Dilution Buffer, and assayed for cross reactivity. No significant cross-reactivity or interference was observed.

PROTEINS	CROSS-REACTIVITY
Human PCSK9 (NS0-derived)	100
Human mature form of PCSK9 (E. Coli-derived)	100
Mouse PCSK9	0
Human PCSK1	0
Human PCSK7	0
Human FABP-4	0
Human ATGL	0
Human FTO	0
Human Vaspin	0

REFERENCES

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3: Dong B, et al.. Strong induction of PCSK9 gene expression through HNF1{alpha} and SREBP2: Mechanism for the resistance to LDL-cholesterol lowering effect of statins in dyslipidemic hamsters. J Lipid Res. 2010 Jan 4. [Epub ahead of print]

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SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARDS
↓
Add 100 µl of standard, samples, positive control each well. Incubate 2 hours on the plate shake at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µl 1x Antibody Working Solution to each well. Incubate 2 hours on the plate shaker at RT.
↓
Add 100 µl Streptavidin-HRP conjugate working solution to all wells. Incubate 60 min on the plate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µl Substrate Solution to each well. Incubate 15-25 min on plate shaker. **Protect from light.**
↓
Add 100 µl Stop Solution to each well. Read 450nm within 15 min