# Human PCSK9 ELISA

REF Cat. No.: BTPCSK9-001

For Quantitative Determination of Human
Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9)

96 Determinations

2°C – 8 °C

For research use only
Not for diagnostic use

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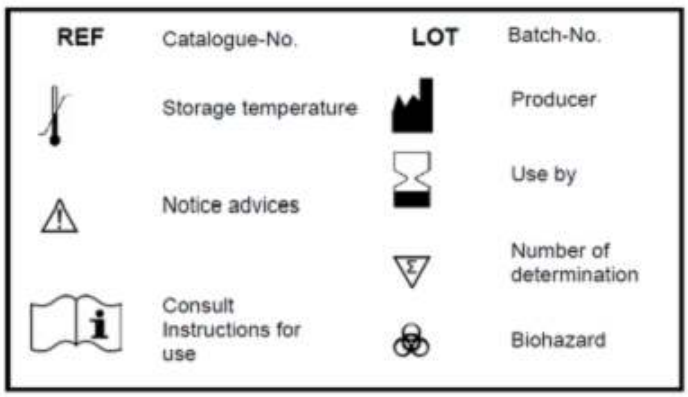
# Contents





## Introduction

Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) has medical importance because it interferes with the lipoprotein in particular the lipoprotein particles (LDL) homeostasis. PCSK9 binds to the lipoprotein particles receptor (LDLR) which transports fat molecules within the extracellular fluid. After LDL binding to the LDL receptor the complex of LDLR/PCSK9/LDL gets degraded in the hepatocytes upon internalization. Importantly if PCSK9 does not bind to the complex, the LDLR can return to the surface of the cell and can thereby continue to remove LDL particles from the bloodstream.

Agents which block PCSK9 can lower LDL particle concentrations in the blood. The therapeutical antibody against PCSK9 Evolocumab, was approved by the U.S. Food and Drug Administration in 2015 for lowering LDL particle concentrations when statins and other drugs were not sufficiently effective or poorly tolerated.

## Intended use

The Human PCSK9 ELISA provides a sensitive and specific quantitative determination of PCSK9 in human serum samples. Do not use for any other biological sample. One kit contains reagents for 96 determinations, thus allowing the measurement of one standard curve and 40 samples in duplicate. The calibration curve covers the range from 5.27 ng/ml to 60 ng/ml. The sensitivity of the assay is 1 ng/ml. The recommended dilution of the serum samples is 1:20. The complete incubation time is 2 hours 20 minutes.

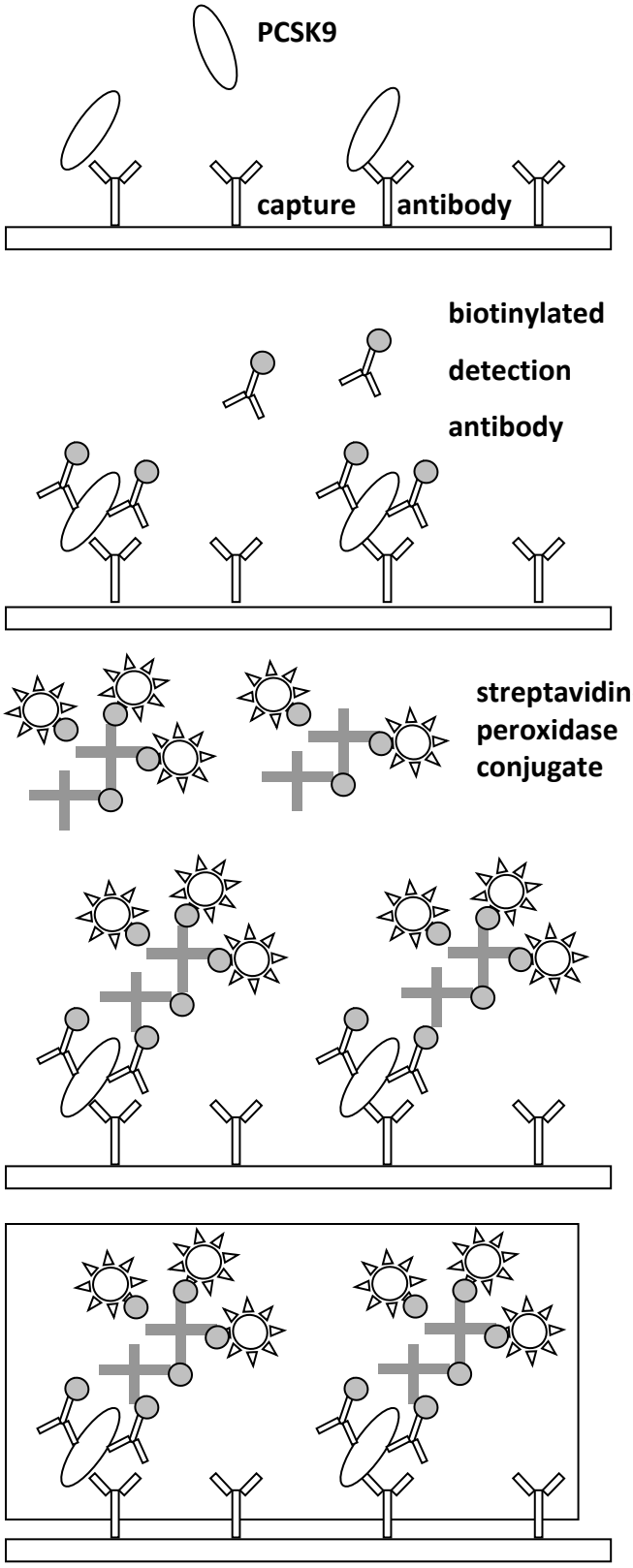
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

## Test principle

The Human PCSK9 ELISA (Enzyme-Linked Immunosorbent Assay) is an immunological quantitative detection method based on a sandwich ELISA.

The ELISA is performed in a 96-well microplate. The wells of the microplate are pre-coated with a specific capture antibody against human PCSK9 that binds free PCSK9 from the patient sample. The analyte is detected in two steps using a biotin-labelled monoclonal antibody and a highly polymerised streptavidin-peroxidase conjugate. The incubation steps take place for 45 min/20 min at 20-25°C. After a washing step, the colour substrate TMB (Tetramethylbenzidine) is added to the wells. The reaction is stopped by adding the stop solution and the yellow colour is read in a microtiter plate reader at 450 nm. The concentration of PCSK9 in a sample is determined by interpolation from the standard curve.

## Principle of the assay




**Step 1:** Incubation of standard or sample on the microtiter plate. Specific binding of PCSK9
(duration: 45 minutes)

**Step 2:** Detection of bound PCSK9 with biotinylated antibody
(duration: 45 minutes)

**Step 3:** Addition of streptavidin-peroxidase conjugate
(duration: 45 minutes)

**Step 4:** Colour development after addition of TMB substrate
(duration: 30 minutes)

**Fig. 1:** Scheme of the assay procedure

## Safety warning and precautions

**Warning**: The kit contains substances such as 1M sulphuric acid and ProClin 300 (maximum 0.04%) as a preservative in reagents that are corrosive and/or toxic. TMB has a reproductive toxity: May damage the unborn child. Avoid contact with eyes and skin! Wear protective gloves!
The **PCSK9-DILUTION-BUF** contains human serum that has been negatively tested for contaminations of HAV, HBV, HCV and HIV. Wear suitable protective clothing, such as laboratory overalls and gloves and observe caution when working with this material. In the event of injury a medical specialist should always be consulted.
All chemicals should be considered as being potentially hazardous. 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. Avoid contact with skin and eyes. In case of skin and eyes contact, wash immediately with water.

## Storage

All components of the kit should be stored in the refrigerator (2-8 °C).
Once reconstituted, the **PCSK9-Standard-CONC** should be used immediately or stored at -80 °C.
The dilutions of **BIOT-ANTIBODY-CONC** and **STAV-CONJ-CONC** should be prepared freshly directly before use. When running a partial plate, only suitable aliquots of these solutions should be made.
After dilution **WASHBUF** is stable for 4 weeks at storage temperature 2-8°C.

## Components of the assay system

| No. | Components | Marking | Volume/ Quantity | Colour of top cap | Con-dition ** |
|---|---|---|---|---|---|
| 1 | Microplate coated with anti-PCSK9 antibody | **PCSK9-PLATE** | 12 Strips á 8 well | - | G |
| 2 | Sample dilution buffer | **PCSK9-SAMPLE-BUF** | 1 x 15.0 mL | colourless | G |
| 3 | PCSK9 Standard concentrate (300 ng PCSK9 lyophilized) | **PCSK9-STANDARD-CONC** | 1 x | purple | R |
| 4 | PCSK9 dilution buffer | **PCSK9-DILUTION-BUF** | 1 x 6.0 mL | blue | G |
| 5 | Biotinylated antibody concentrate | **BIOT-ANTIBODY-CONC** | 1 x 0.15 mL | yellow | R |
| 6 | Streptavidin-peroxidase conjugate concentrate | **STAV-CONJ-CONC** | 1 x 0.1 mL | black | R |
| 7 | Buffer | **BUFFER** | 1 x 30.0 mL | green | G |
| 8 | TMB reagent | **TMB** | 1 x 12.0 mL | brown | G |
| 9 | Stopping solution (1M $H_2SO_4$) | **STOP-$H_2SO_4$** | 1 x 4.0 mL | colourless | G |
| 10 | Washing buffer concentrate (10x) | **WASHBUF 10x** | 2 x 30.0 mL | colourless | R |
| 11 | Sealing foil for microplate | **-** | 1 piece | - | G |

*** Condition: G = ready for use; R = reconstitution (dilution) required**

**PCSK9-PLATE:** The plate contains 12 strips (96 wells) coated with monoclonal anti-PCSK9 antibody. Ready to use.
**PCSK9-SAMPLE-BUF**: The bottle contains 15 ml buffer containing additives. Ready to use.
**PCSK9-STANDARD-CONC** (lyophilized): The vial contains 300 ng of lyophilized PCSK9. It has to be reconstituted with 1 ml of destilled water prior to use to get a 300 ng/ml standard solution.
**PCSK9-DILUTION-BUF**: Solution to produce PCSK9-STANDARDS after reconstitution PCSK9-STANDARD-CONC (lyophilized). Ready to use.
**BIOT-ANTIBODY-CONC**: Solution (150 µl) contains biotinylated monoclonal anti-PCSK9 antibody in buffer with additives. The antibody has to be diluted 100-fold with BUFFER before use.
**STAV-CONJ-CONC:** Solution (100 µl) contains a highly polymeric streptavidin-peroxidase conjugate with preservatives. It has to be diluted 333-fold with BUFFER prior to use.
**BUFFER**: Solution (30 ml) consists of phosphate buffer containing additives. Ready to use.
**WASHBUF 10x:** Each bottle contains 30 ml buffer concentrate. It has to be diluted 10-fold with distilled or deionised water before use.
**TMB:** The bottle contains 12 ml of a TMB solution. Ready to use.
**STOP-$H_2SO_4$:** The bottle contains 4 ml 1 M $H_2SO_4$. Ready to use. **Warning**: Stop solution contains 1 M sulfuric acid. Wear eye, hand, face and clothing protection when using this material!

**EQUIPMENT REQUIRED BUT NOT PROVIDED**

- Pipettes with disposable tips (50 µl, 100 µl and 1 ml), a multi-channel pipette (100 µl) would be appropriate
- Distilled or deionised water
- Horizontal orbital microplate shaker
- Microplate reader capable of measuring at 450 nm

## Sample preparation and storage

**Serum:**

- Serum may be stored at -80°C. When stored at -80°C, it is absolutely necessary to mix the samples thoroughly prior to measuring. Avoid freeze-thaw cycles.
- Dilute the serum samples minimum **1:20** with PCSK9-SAMPLE-BUF, depending on the possible concentration of the analyte.

## Critical Parameters

- Allow samples and all reagents to equilibrate to room temperature (20-25°C) prior to performing the assay. This is especially a prerequisite for reconstituted WASHBUF and TMB!
- It is absolutely important that all wells are washed thoroughly and uniformly. When washing is done by hand, ensure that all wells are completely filled and emptied at each step by discarding the content of the plate. Carefully tap off excess liquid (see technical advice on the following link: http://www.youtube.com/watch?v=FZirnCas17Y)
- Use only reagents from the same lot for each assay. This is especially important when running more than one plate per sample group.

- A separate standard curve must be run on each plate.
- Mix all reagents thoroughly prior to use, but **avoid foaming**!
- Keep the wells sealed with the foil except when adding reagents and during reading.
- Any variation in the protocol can cause variation in binding!
- The kit should not be used beyond the expiration date on the kit label.
- The values obtained by the samples should be within the standard range. If this is not the case, dilute the sample and repeat the assay.
- We take great care to ensure that this product is suitable for the validated sample type, as designated in this manual. However, it is possible that in some cases, high levels of interfering substances may cause unusual results.

## Preparation of reagents

Please note: To prevent edge effects it is necessary to equilibrate all reagents **to room temperature** prior to use. For the dilution of the WASHBUF 10x use either distilled or deionised water. Always seal the plates with the provided foil during incubation!

**PCSK9-PLATE:** After equilibration to room temperature the plate is ready to use. The plate should be removed from its packaging immediately before start of pipetting.
**PCSK9-SAMPLE-BUF**: After equilibration to room temperature the buffer is ready to use.
**PCSK9-STANDARD-CONC** (lyophilized): Add 1 ml destilled water to the standard tube (purple lid) and allow the contents to dissolve for 5-10 minutes. Gently mix, but avoid foaming of the reagent!
**PCSK9-DILUTION-BUF**: After equilibration to room temperature the reagent is ready to use.
**BIOT-ANTIBODY-CONC:** After equilibration to room temperature dilute this reagent 100-fold with BUFFER immediately before use. For a whole plate add 120 µl from the BIOT-ANTIBODY-CONC (yellow lid) to 12 ml BUFFER (green lid). When running half a plate, add 60 µl BIOT-ANTIBODY-CONC (yellow lid) to 6 ml BUFFER (green lid).
**STAV-CONJ-CONC**: After equilibration to room temperature dilute this reagent 333-fold with BUFFER immediately before use. For a whole plate add 36 µl from the STAV-CONJ-CONC (black lid) to 12 ml BUFFER (green lid). When running half a plate, add 18 µl STAV-CONJ-CONC (black lid) to 6 ml BUFFER (green lid).
**WASHBUF 10x:** After equilibration to room temperature dilute the WASHBUF 10x concentrate 1:10 with distilled or deionised water (for example: 25 ml WASCHBUF 10x to 250 ml).
**TMB and STOP-$H_2SO_4$:** After equilibration to room temperature the reagents are ready to use.

### Preparation of PCSK9 standards with PCSK9-DILUTION-BUF

1. Label 7 tubes with 5.3, 7.9, 11.9, 17.8, 26.7, 40 and 60 ng/ml.
2. Pipette 600 µl of PCSK9-DILUTION-BUF into the 60 ng/ml tube, in the remaining tubes (5.3, 7.9, 11.9, 17.8, 26.7, 40 ng/ml) pipette 250 µl of PCSK9-DILUTION-BUF.
3. Pipette 150 µl of the diluted PCSK9-STANDARD-CONC (300 ng/ml) into the 60 ng/ml tube and mix thoroughly.
4. Pipette 500 µl of the 60 ng/ml standard into the tube labelled with 40 ng/ml and mix thoroughly.
5. Repeat this dilution procedure with the other standard tubes.
6. The blank value (0 ng/ml) is obtained by using only PCSK9-DILUTION-BUF.

7. The stock solution is not part of the standard curve and can be stored at -80°C. Avoid freeze-thaw cycles.

## Assay protocol

1. Prepare reagents and standards as described in the sections above. **Remember that it is necessary to equilibrate the reagents to room temperature before use**.
2. Prepare the samples as described above by appropriate dilution with PCSK9-SAMPLE-BUF. Recommended Dilution of the serum samples 1:20 (for example: 15 µl sample to 285 µl PCSK9-SAMPLE-BUF)
3. Prepare the microtiter plate by inserting the required amount of wells into the frame. **Note that you need 16 wells for the standard curve**.
4. **Standard curve:** Pipette 100 µl of the reconstituted standards 5.3, 7.9, 11.9, 17.8, 26.7, 40 and 60 ng/ml in duplicate in the wells using a clean pipette tip for each standard. PCSK9-Dilution-BUF serves as zero blank.
5. **Samples:** Pipette 100 µl of the diluted samples in duplicate into the wells.
6. Seal the plate with the provided foil and incubate on a shaker at room temperature (20-25°C) for exactly 45 minutes. Keep the plate in the dark.
7. Wash by filling each well with diluted WASHBUF (300 µl/well), then remove by discarding/drying by tapping inverted plate on clean paper towels. Take care that all wells are completely filled and emptied at each washing step. Wash the wells 3 times with diluted WASHBUF.
8. Add 100 µl of diluted Biotinylated Antibody (BIOT-ANTIBODY-CONC) into each well.
9. Seal the plate and incubate on a shaker at room temperature for exactly 45 minutes.
10. Wash by filling each well with diluted WASHBUF (300 µl/well), then remove by discarding/drying by tapping inverted plate on clean paper towels. Take care that all wells are completely filled and emptied at each washing step. Wash the wells 3 times with diluted WASHBUF.
11. Add 100 µl of diluted Streptavidin-conjugate solution (STAV-CONJ-CONC) into each well.
12. Seal the plate with the provided foil and incubate on a shaker at room temperature for exactly 20 minutes.
13. Wash by filling each well with diluted WASHBUF (300 µl/well), then remove by discarding/drying by tapping inverted plate on clean paper towels. Take care that all wells are completely filled and emptied at each washing step. Wash the wells 3 times with diluted WASHBUF.
14. Add 100 µl of TMB solution to each well.
15. Seal the plate with foil provided and incubate in the dark at room temperature on a shaker for 30 minutes.
16. Stop the reaction by adding 25 µl of STOP-$H_2SO_4$ to each well.
17. Read the plate at 450 nm (620 nm reference filter). Reading of the plate without reference may yield higher absorbance and thus may be less accurate.

## Protocol summary

Prepare reagents, standards and samples as described, equilibrate reagents to **room temperature.**

Pipette 100 µl standard or sample in duplicate into the wells. Incubate 45 min on a shaker.

Discard/Dry by tapping/Wash 3 times

Add 100 µl of diluted Biotinylated Antibody to each well. Incubate 45 minutes at room temperature on a shaker.

Discard/Dry by tapping/Wash 3 times

Add 100 µl of diluted Streptavidin-conjugate solution to each well. Incubate for 20 minutes at room temperature on a shaker.

Discard/Dry by tapping/Wash 3 times

Add 100 µl of TMB substrate to each well. Incubate for 30 minutes at room temperature in the dark on a shaker!

Add 25 µl STOP-$H_2SO_4$ solution to each well. Read at 450 nm (with reference filter at 620 nm).

## Scheme of the plate

| Strip | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Row | **Field of PCSK9 calibrators** | | **Field of diluted samples** | | | |
| A | PCSK9-Standard 0 ng/ml | PCSK9-Standard 0 ng/ml | Sample 1 | Sample 1 | Sample 9 | Sample 9 |
| B | PCSK9-Standard 5.3 ng/ml | PCSK9-Standard 5.3 ng/ml | Sample 2 | Sample 2 | Sample 10 | Sample 10 |
| C | PCSK9-Standard 7.9 ng/ml | PCSK9-Standard 7.9 ng/ml | Sample 3 | Sample 3 | Sample 11 | Sample 11 |
| D | PCSK9-Standard 11.9 ng/ml | PCSK9-Standard 11.9 ng/ml | Sample 4 | Sample 4 | Sample 12 | Sample 12 |
| E | PCSK9-Standard 17.8 ng/ml | PCSK9-Standard 17.8 ng/ml | Sample 5 | Sample 5 | Sample 13 | Sample 13 |
| F | PCSK9-Standard 26.7 ng/ml | PCSK9-Standard 26.7 ng/ml | Sample 6 | Sample 6 | Sample 14 | Sample 14 |
| G | PCSK9-Standard 40 ng/ml | PCSK9-Standard 40 ng/ml | Sample 7 | Sample 7 | Sample 15 | Sample 15 |
| H | PCSK9-Standard 60 ng/ml | PCSK9-Standard 60 ng/ml | Sample 8 | Sample 8 | Sample 16 | Sample 16 |
| Strip | 1 | 2 | 3 | 4 | 5 | 6 |

Example of the layout for pipetting PCSK9 standards and samples on the plate

## Data processing

### Calculation of results

The calculation is illustrated using representative data: the assay data should be similar to that shown in table 1.

1. Calculate the average absorbance for each set of standard wells.
2. A standard curve is generated by plotting the mean absorbance (x-axis, fig. 2) against ng/ml standard (y-axis, fig.2).
3. The ng/ml values of the samples can be read directly from the graph or calculated by the regression coefficients.
4. Multiply the calculated ng/ml values by the dilution factor of the samples.

| PCSK9 Standard (ng/ml) | PCSK9 Standard curve Absorbance (450 nm) | | |
|---|---|---|---|
| | Value 1 | Value 2 | Mean Value |
| 0.00 | 0.032 | 0.031 | 0.032 |
| 5.3 | 0.208 | 0.213 | 0.211 |
| 7.9 | 0.319 | 0.341 | 0.330 |
| 11.9 | 0.535 | 0.512 | 0.524 |
| 17.8 | 0.764 | 0.842 | 0.803 |
| 26.7 | 1.224 | 1.268 | 1.246 |
| 40.0 | 1.848 | 1.782 | 1.815 |
| 60.0 | 2.405 | 2.444 | 2.425 |

**Table 1:** Typical assay data

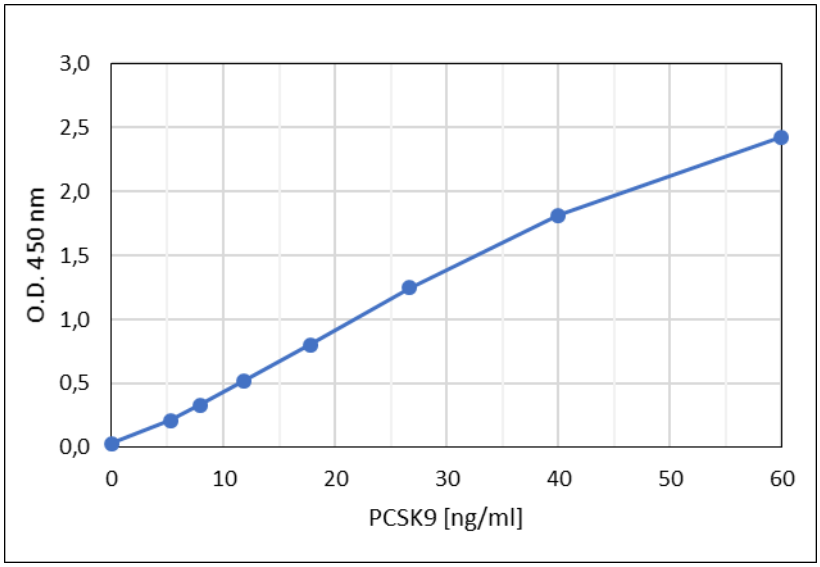



**Fig. 2:** Typical PCSK9 standard curve

## Additional Information

### Specificity

The PCSK9 ELISA has a high sensitivity and high specificity for quantitative determination of human PCSK9. The reactivity with PCSK9 of other species was not tested. Cross-reactions with other serum proteins cannot be verified in physiological concentrations.

### Sensitivity

The minimum detectable dose of human PCSK9 is 1 ng /ml. The lower limit of detection was defined as the lowest protein concentration that could be differentiated from zero. The mean O.D. value of 20 replicates of the zero standard added by their three standard deviations was determined.

### Linearity

The assay should be conducted with a serum dilution of 1:20. For dilutions of 1:20 to 1:40, the linearity of the assay results to their dilution is assured.

### Recovery

The recovery of PCSK9 standard spiked to levels throughout the range of the assay in serum was evaluated.

| Matrix | Recovery range (%) |
|---|---|
| Serum (n=5) | 98-112% |

### Reproducibility

*Intra-assay precision*

The intra-assay precision was measured by assaying 3 serum samples with different levels of the antigen PCSK9 on 1 plate, 8 replicates of each sample.

| Sample | Mean value PCSK9 [ng/mL] | CV (%) | N |
|---|---|---|---|
| 1. | 215 | 6.1 | 8 |
| 2. | 449 | 4.0 | 8 |
| 3. | 500 | 4.5 | 8 |

*Inter-assay precision*

The inter-assay variation was measured by assaying 3 serum samples with different levels of the antigen PCSK9 on 3 plates, 8 replicates in each plate.

| Sample | Mean value PCSK9 [ng/mL] | CV (%) | N |
|---|---|---|---|
| 1. | 215 | 12.5 | 24 |
| 2. | 449 | 6.0 | 24 |
| 3. | 500 | 5.0 | 24 |

## Troubleshooting

| Problem | Potential cause | Recommendation |
|---|---|---|
| **Low absorbance** | • Wrong wavelength<br>• Enzyme conjugate out of date/reagents improperly stored<br>• Improper incubation time and temperature<br>• Reagents not equilibrated to RT<br>• Reagents not correctly prepared | • Check reader wavelength<br>• Control the expiration date/storage conditions<br>• Control the incubation time and temperature<br>• Check equilibration of reagents to RT<br>• Check preparation of reagents |
| **High absorbance/ high zero standard value** | • Incomplete washing<br>• Improper removing of residual fluid<br>• Improper incubation time and temperature<br>• Reagents not equilibrated to RT<br>• Reagents not correctly prepared | • Ensure that every well is completely filled/emptied during each washing step<br>• Check that plates are blotted on tissue paper after each washing step<br>• Control the incubation time and temperature<br>• Check equilibration of reagents to RT<br>• Check preparation of reagents |
| **Flat curve/poor reproducibility** | • Wrong wavelength<br>• Enzyme conjugate out of date/reagents improperly stored<br>• Improper preparation of working standards<br>• Pipette errors<br>• Contamination of components by use of unclean reservoirs/used pipette tips<br>• Edge effects because of using of cold substrate solution<br>• Washing incomplete | • Check wavelength<br>• Control the expiration date/storage conditions<br>• Check preparation of standards<br>• Check pipette calibration<br>• Use separate reservoirs and always new pipette tips<br>• Equilibrate substrate to room temperature<br>• Ensure sufficient washing procedure |

## Related Products

For detection of human PCSK9 during therapy with the antibody Evolocumab you can use our *recovery*ELISA Kit RPE:
The recoveryELISA RPE kit allows the simultaneous quantitative determination of free PCSK9, the PCSK9 neutralization rate and the available therapeutic antibody Evolocumab in human serum samples. This test should only be used for patients treated with Evolocumab as mono biological therapy. It is a manual, non-automated kit for the determination of 7 samples.

Cat.No.: R777 *recovery*ELISA PCSK9 Neutralization Rate/Evolocumab (RPE)

More information you will find in our homepage: see to http://www.biotez.de/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