ab285349 – Mouse PCSK9 ELISA Kit

For the quantitative detection of PCSK9 in mouse serum, plasma, and cell culture supernatants.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285349

Storage and Stability

Store kit at 4°C for 6 months or at -20°C for 12 months.

Materials Supplied

Item	Quantity	Storage Condition
PCSK9 mAb coated plate, 96 wells	8 x 12 wells	4°C
Mouse PCSK9 standard (10 ng/vial)	2 vials	4°C
Sample diluent buffer	30 mL	4°C
Biotinylated anti-mouse PCSK9 Ab	130 µL	4°C
Antibody diluent buffer	12 mL	4°C
Avidin-Biotin-Peroxidase Complex (ABC)	130 µL	4°C
ABC diluent buffer	12 mL	4°C
TMB (Colorless)	10 mL	4°C
Stop Solution	10 mL	4°C
Wash buffer Powder	1 pack	4°C
Plate sealers	4 units	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm.
- Absorbent paper.
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.

Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.

Mouse PCSK9 standard: Two vials of PCK9 standard (10 ng per vial) are included in each kit. Use one vial for each experiment. Prepare 10,000 pg/ml of mouse PCSK9 standard solution by adding 1ml of sample diluent buffer into one of the vials. Keep the tube at room temperature for 10 min. and mix thoroughly. Label 6 Eppendorf tubes with 5000 pg/ml, 2500 pg/ml, 1250 pg/ml, 625 pg/ml, 312 pg/ml & 156 pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the 10,000 pg/ml PCK9 standard solution into 1st tube and mix. Transfer 0.3 ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Δ Note: *The standard solutions are best used within 2 hrs. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hrs, or at - 20°C for up to 48 hrs. Avoid repeated freeze-thaw cycles*

Biotinylated anti-mouse PCSK9 antibody working solution: Dilute 1:100 with the antibody diluent buffer and mix thoroughly. Prepare 0.1 ml of PCSK9 antibody working solution for each well. Solution should be prepared no more than 2 hrs. prior to the experiment.

Avidin-Biotin-Peroxidase Complex (ABC) working solution: Dilute 1:100 with the ABC dilution buffer and mix thoroughly. Prepare 0.1 ml of ABC working solution for each well. Solution should be prepared no more than 1 hr. prior to the experiment.

Wash Buffer: Dissolve the wash buffer powder in 1000 ml of water to make 1X PBS wash buffer.

Sample Preparation

Samples to be used within 5 days may be stored at 4°C, otherwise samples must be stored at -20°C (≤1 month) or -80°C (≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles.

Serum:
Allow the serum to clot in a serum separator tube (about 4 hrs) at room temperature. Centrifuge at approximately 2000 X g for 15 min. Analyze the serum immediately or aliquot and store at -20°C.

Plasma:
Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min. at 2000 x g within 30 min. of collection. Analyze immediately or aliquot and store frozen at -20°C. Citrate is not recommended as an anticoagulant.

Cell culture supernatant:
Centrifuge cell culture supernates to remove particulates, assay immediately or aliquot and store at -20°C.

Δ Notes:

a) *Store samples to be assayed within 24 hrs. at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.*

b) *Sample dilution guidelines: The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The sample must be well mixed with the diluents buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. For high target protein concentration (100-1000 ng/ml): dilute 1:100. For medium target protein concentration (10-100 ng/ml): dilute 1:10. For low target protein concentration (156-10,000 pg/ml): dilute 1:2. For very low target protein concentration (≤156 pg/ml). No dilution necessary or dilute 1:2.*

Assay Protocol

The ABC working solution and TMB reagent must be kept warm at 37°C for 30 min. before use.
When diluting samples and reagents, they must be mixed completely and evenly.
Don't let 96-well plate dry, as it will inactivate active components on plate.
Prepare all reagents, samples and standards.

1. Standard preparation: Number tubes 1-8. Final Concentrations to be 1-4000pg/ml, 2-2000pg/ml, 3-1000pg/ml, 4-500pg/ml, 5-250pg/ml, 6-125pg/ml, 7- 62.5pg/ml, 8-0.0 (Blank).
    a. For standard #1, add 1000 µl of undiluted standard stock solution to tube #1.
    b. Add 300 µl of sample diluent to tubes # 2-7.
    c. To generate standard #2, add 300 µl of standard #1 from tube #1 to tube #2 for a final volume of 600 µl. Mix thoroughly.
    d. To generate standard #3, add 300 µl of standard #2 from tube #2 to tube #3 for a final volume of 600 µl. Mix thoroughly.
    e. Continue the serial dilution for tube #4-7.
    f. Tube #8 is a blank standard to be used with every experiment.

    Aliquot 0.1ml per well of each mouse PCK9 standard solutions into the precoated 96-well plate. Add 0.1ml of the sample diluent buffer into the control well (Zero well). Add

0.1ml of each properly diluted sample of mouse cell culture supernatants, serum or plasma (heparin, EDTA) to each empty well.

Δ Notes:

a) *We recommend that each mouse PCK9 standard solution and each sample is measured in duplicate.*

b) *We recommend doing a pilot experiment using standards and a small number of samples to inspect the validity of experiment operation and the appropriateness of sample dilution proportion.*

2. Seal the plate with the cover and incubate at 37°C for 90 min. Remove the cover, discard plate content, and blot the plate onto paper towels or other absorbent material. Do not let the wells completely dry at any time.
3. Add 0.1ml of biotinylated anti-mouse PCK9 antibody working solution into each well and incubate the plate at 37°C for 60 min. Wash plate 3 times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (Plate Washing Method: Discard the solution in the plate without touching the side walls. Blot the plate onto paper towels or other absorbent material. Soak each well with at least 0.3 ml PBS or TBS buffer for 1~2 min. Repeat this process two additional times for a total of three washes. Note: For automated washing, aspirate all wells and wash three times with PBS or TBS buffer, overfilling wells with PBS or TBS buffer. Blot the plate onto paper towels or other absorbent material.)
4. Add 0.1ml of prepared ABC working solution into each well and incubate the plate at 37°C for 30 min. Wash plate 5 times with 0.01M TBS or 0.01 M PBS, and each time let washing buffer stay in the wells for 1-2 min. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 3 for plate washing method).
5. Add 90 µl of prepared TMB reagent into each well and incubate plate at 37°C in dark for 20-25 min. Note: For reference only, the optimal incubation time should be determined by end user. The shades of blue can be seen in the wells with the four most concentrated mouse PCK9 standard solutions; the other wells show no obvious color.
6. Add 0.1ml of prepared Stop solution into each well. The color changes into yellow immediately.
7. Read the O.D. absorbance at 450 nm in a microplate reader within 30 min. after adding the stop solution.

Calculation:

Relative O.D.450 = O.D.450 of each well – O.D.450 of Zero well. The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The mouse PCK9 concentration of the samples can be interpolated from the standard curve.

Δ Note: if the samples were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

For technical support contact information, visit: www.abcam.com/contactus