Description
The PCSK9(His)-LDLR Binding Assay Kit is an ELISA-type 384-well format assay designed for screening and profiling purposes. The Assay Kit contains His-tag PCSK9, purified LDLR ectodomain and Anti-His-tag Horseradish Peroxidase (HRP)-conjugated antibody in sufficient amount for 400 binding reactions. Moreover, two pre-formulated assay buffers are supplied to validate PCSK9-LDLR binding affinity in either neutral or acidic binding conditions. The assay takes advantage of the high sensitivity of detection of His-tag PCSK9 by Anti-His HRP-antibody. First, LDLR ectodomain is coated on a 384-well plate. Next, PCSK9 is incubated with LDLR on the plate. Finally, the plate is treated with Anti-His HRP-antibody followed by addition of an HRP substrate to produce chemiluminescence, measured using a chemiluminescence reader.

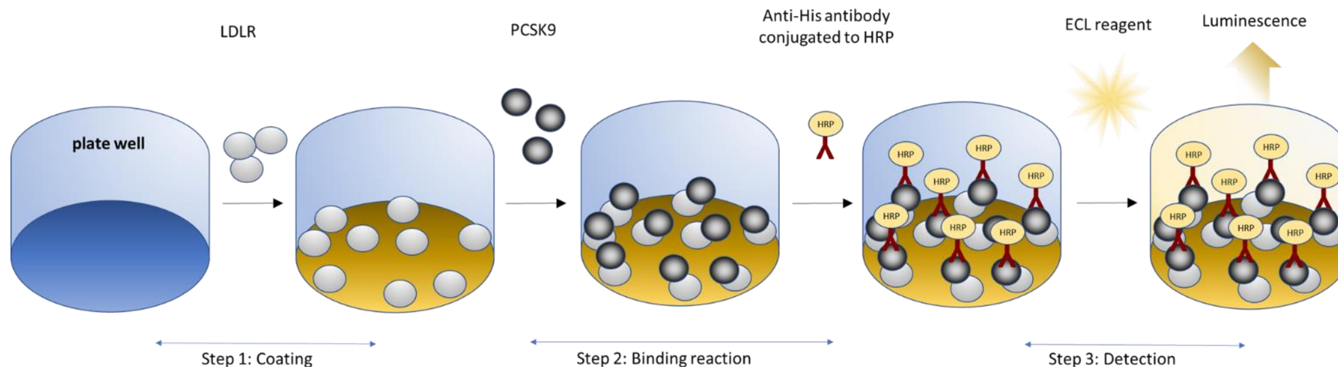

Figure 1: Illustration of the ELISA-based PCSK9(His)-LDLR Binding Assay Kit.

Background
PCSK9 (Proprotein convertase subtilisin/kexin type 9) functions as a negative regulator of hepatic low-density lipoprotein receptors (LDLRs) and therefore is a critical regulator of cholesterol metabolism. It is an endopeptidase that binds to the EGFR-like ectodomain of LDLR, leading to LDLR degradation, which in turn, results in increased circulating LDL. Inhibiting the PCSK9-LDLR interaction is an increasingly desirable therapeutic approach for lowering LDL-cholesterol levels to replace or supplement statins. New therapies are critical for addressing atherosclerosis, stroke, heart disease, and other cardiovascular disorders.

Applications
Screen small molecules and antibodies that inhibit the binding of PCSK9 to LDLR ectodomain.

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Supplied Materials

<table>
<tr><th>Catalog #</th><th>Name</th><th>Amount</th><th>Storage</th><th></th></tr>
<tr><td>71204</td><td>PCSK9(His)*</td><td>2 x 10 µg</td><td>-80°C</td><td rowspan="8">Avoid multiple freeze/ thaw cycles</td></tr>
<tr><td>71205</td><td>LDLR*</td><td>2 x 10 µg</td><td>-80°C</td></tr>
<tr><td></td><td>Anti-His-HRP-Antibody-D</td><td>2 x 10 µl</td><td>-80°C</td></tr>
<tr><td>33298</td><td>3x PL-01 Assay Buffer</td><td>2 x 50 ml</td><td>-20°C</td></tr>
<tr><td>79727</td><td>3x PL-02 Assay Buffer</td><td>2 x 50 ml</td><td>-20°C</td></tr>
<tr><td>79728</td><td>Blocking Buffer 2</td><td>2 x 50 ml</td><td>+4°C</td></tr>
<tr><td rowspan="2">79670</td><td>ELISA ECL Substrate A (translucent bottle)</td><td>2 x 6 ml</td><td>Room Temp</td></tr>
<tr><td>ELISA ECL Substrate B (brown bottle)</td><td>2 x 6 ml</td><td>Room Temp</td></tr>
<tr><td>78188</td><td>384-well plate</td><td>1</td><td>Room Temp</td><td></td></tr>
</table>

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- PBS buffer (Phosphate Buffer Saline)
- Luminometer or microplate reader capable of reading chemiluminescence
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/ thaw cycles!***

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

PCSK9(His)-LDLR Binding Assay Kit is compatible with up to 1% final DMSO concentration. We recommend preparing the inhibitor in no higher than 10% DMSO solution in buffer and using 5 µl per well.

Assay Protocol

- All samples and controls should be performed in duplicates.
- The assay should include a “Blank” “Uncoated control” and a “Positive control”.

Coating the plate with LDLR:

1) Choose the neutral or acidic buffer at which you wish to test the interaction between PCSK9 and LDLR. **You can either run the reaction using acidic PL-01 Assay Buffer (pH 6.0) or neutral PL-02 Assay Buffer (pH 7.4)**.

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2) Thaw LDLR on ice. Briefly spin the tube containing LDLR to recover the full contents of the tube. If the assay plate is going to be used more than once, calculate the amount of protein required for this portion of the assay. Aliquot the remaining undiluted protein into single use aliquots depending on how many times the plate will be used and immediately store the aliquots at -80°C.

3) Dilute LDLR to 2 ng/µl in PBS (enough for 25 µl/well).

4) Add 25 µl of diluted LDLR to each well except "Uncoated Control" and incubate overnight at 4°C. *Do not freeze and reuse the diluted protein.*

5) The following day, dilute the 3x Assay Buffer of your choice to 1x Assay Buffer by adding one volume of 3x Assay Buffer to two volumes of distilled water.

6) Wash the plate 3 times with 50 µl/well of 1x Assay Buffer. Tap the plate onto clean paper towels to remove the excess liquid.

7) Block by adding 50 µl of Blocking Buffer 2 to each well. Incubate for 1 hour at room temperature. Remove the Blocking Buffer.

Step 1:

1) Prepare the Master Mix: N wells x (5 µl of 3x Assay Buffer + 7.5 µl of distilled water)

2) Add 12.5 µl of Master Mix to each well, including "Blank", "Uncoated control", "Positive Control" and "Test Inhibitor".

3) Prepare the Test Inhibitor (2.5 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 25 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in 1x Assay buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.

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4) Add 2.5 µl of inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control," "Uncoated Control" and "Blank," add 2.5 µl of Diluent Solution (for example 10% DMSO in 1x Assay Buffer).

5) Add 10 µl of 1x Assay Buffer to the well designated "Blank."
6) Thaw PCSK9(His) on ice. Briefly spin the tube containing the protein to recover full the contents of the tube. Calculate the amount of protein required for the assay and dilute enough for the assay (25 ng/well).

   Aliquot unused protein into 2-4 aliquots as may be necessary (single use aliquots) and store them at -80°C.

   Note: The protein is sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles and do not reuse the diluted protein.

7) Initiate the reaction by adding 10 µl/well of diluted PCSK9(His) to "Uncoated control", "Positive Control" and "Test Inhibitor" (do NOT add to "Blank"). Incubate at room temperature for two hours.

	Blank	**Uncoated Control**	**Positive Control**	**Test Inhibitor**
Master Mix	12.5 µl	12.5 µl	12.5 µl	12.5 µl
Test Inhibitor	–	–	–	2.5 µl
Diluent Solution (no Inhibitor)	2.5 µl	2.5 µl	2.5 µl	–
1x Assay Buffer	10 µl	–	–	–
PCSK9(His) (2.5 ng/µl)	–	10 µl	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

8) Wash the plate 3 times with 50 µl/well of 1x Assay Buffer. Tap the plate onto clean paper towels to remove the liquid.

9) Block wells by adding 50 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Remove the liquid from the plate.

Step 2:

1) Dilute Anti-His-HRP-D antibody 1000-fold with Blocking Buffer 2.

2) Add 50 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3) Wash the plate three times with 50 µl/well of 1x Assay Buffer. Tap the plate onto clean paper towels to remove the liquid.

4) Block by adding 50 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Remove the Blocking Buffer and tap the plate onto clean paper towels to remove the liquid.

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Step 3:

1) Just before use, mix N wells x (25 µl of ELISA ECL substrate A and 25 µl/well of ELISA ECL substrate B), then add 50 µl of the mix to each well. Discard any unused chemiluminescent mix after use.

2) Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence. The "Blank" value is subtracted from all readings.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry. To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

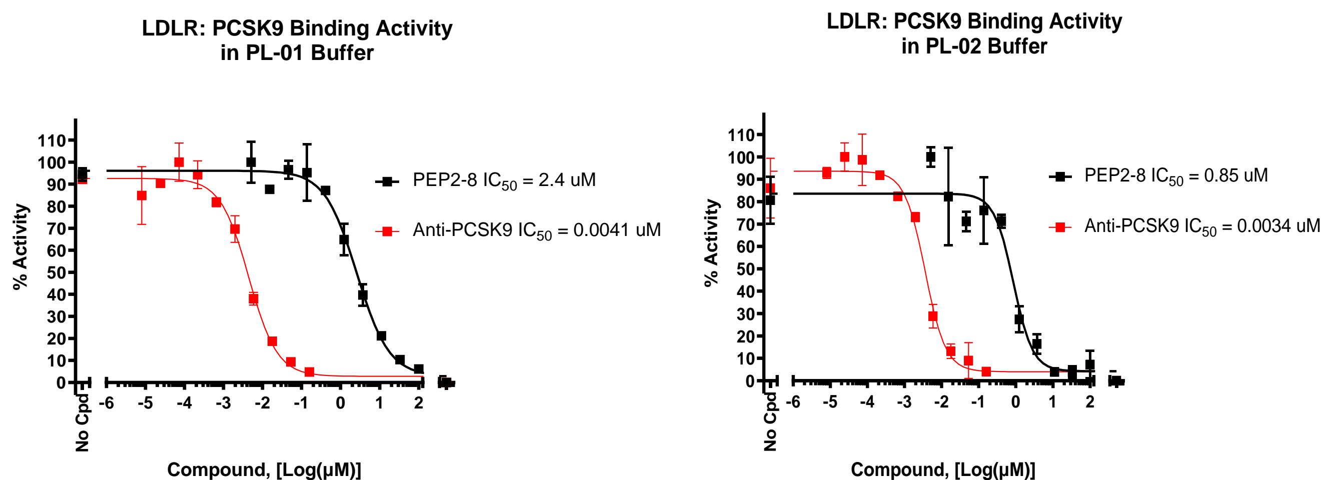

Figure 2: PCSK9-LDLR binding activity in neutral or acidic buffer.

PCSK9-LDLR binding activity was measured using the PCSK9(His)-LDLR Binding Assay Kit (BPS Bioscience #78813) using two different buffers in the presence of increasing concentrations of Anti-PCSK9 Inhibitor (BPS Bioscience #71207) and PEP2-8 (MedChemExpress #HY-P2276). Luminescence was measured using a Bio-Tek microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

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Related Products:

Products	*Catalog #*	*Size*
PCSK9-LDLR Homogeneous Assay Kit	78812	384 reactions
PCSK9, C-terminal His-Avi-Tag, Biotin-Labeled Recombinant	71304	25 µg
LDLR, FLAG-tag Recombinant	71205	50 µg
LDLR, Biotin-Labeled Recombinant	71206	25 µg
Anti-PCSK9 Neutralizing Antibody	71207	50 µg
PCSK9-LDLR TR-FRET Assay Kit	72010	384 reactions
PCSK9(D374T)-LDLR TR-FRET Assay Kit	72011	384 reactions

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