Description

The PCSK9(D374Y) [Biotinylated]-LDLR Binding Assay Kit is designed for screening and profiling purposes. The kit comes in a convenient 96-well format and contains enough biotin-labeled mutant protein PCSK9(D374Y), purified LDLR ectodomain, and HRP-conjugated streptavidin for 100 reactions. Moreover, two pre-formulated assay buffers are supplied to validate PCSK9-LDLR binding in either neutral or acidic binding conditions.

This assay takes advantage of the high sensitivity of detection of biotin-labeled PCSK9(D374Y) by streptavidin-HRP. First, a 96-well plate is coated with LDLR ectodomain. PCSK9(D374Y) is then incubated with LDLR. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which is measured using a chemiluminescence reader.

Background

PCSK9 (Proprotein convertase subtilisin/kexin type 9) functions as a negative regulator of the hepatic low-density lipoprotein receptors (LDLRs) by binding to the LDLR ectodomain. The D374Y mutation is associated with severe hypercholesterolemia; this form of PCSK9 is more potent at decreasing LDL uptake than wild-type PCSK9, most likely by increasing the binding affinity of PCSK9 for LDLR.

Applications

Screen small molecules and antibodies that inhibit the binding of PCSK9(D374Y) to the LDLR ectodomain in high-throughput screening applications (HTS).

Supplied Materials

Catalog #	Name	Amount	Storage
101725	PCSK9(D374Y), Biotin-Labeled*	10 µg	-80°C
71205	LDLR, FLAG-Tag*	10 µg	-20°C
79742	Streptavidin-HRP	10 µl	-20°C
33298	3x PL-01 Assay Buffer	50 ml	-20°C
79727	3x PL-02 Assay Buffer	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79670	ELISA ECL Substrates A and B (2 components)	6 ml each	Room Temp.
79699	96-well plate, white	1	+4°C

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

Materials Required but Not Supplied

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

Stability

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!***

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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 and using 5 µl per well.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include a “Blank”, “Uncoated Control”, “Positive Control” and “Test Compound”.
- The plate should be coated the day before the assay is to be run.
- If the assay plate is going to be used more than once, calculate the amount of protein and other reagents required for this portion of the assay. Aliquot the remaining undiluted protein and reagents into single use aliquots depending on how many times the plate will be used and immediately store the aliquots at -80°C or -20°C as appropriate.

Step 1: Coat 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)**.

2. Thaw **LDLR** on ice. Briefly spin the tube containing LDLR to recover the full content of the tube.

3. Dilute LDLR to 2 ng/µl in PBS (50 µl/well).

   Note: Do not freeze and reuse the diluted protein.

4. Add 50 µl of diluted LDLR to each well, except the “Uncoated Control” wells, and incubate overnight at 4°C.

5. The following day, dilute the **3x Assay Buffer** of your choice (PL-01 or PL-02) with distilled water to make 1x Assay Buffer.

6. Remove the LDLR coating solution from the plate and wash the plate 3 times with 100 µl/well of 1x Assay Buffer. Tap the plate onto clean paper towels to remove the liquid.

7. Add 100 µl of **Blocking Buffer 2** to each well.

8. Incubate for 1 hour at Room Temperature.

9. Remove the Blocking Buffer from the plate and wash the plate 3 times with 100 µl/well of 1x Assay Buffer. Tap the plate onto clean paper towels to remove the liquid.

Step 2:

1. Add 25 µl of 1x Assay Buffer to the "Positive control", "Test Compound", and "Uncoated Control".

2. Add 45 µl of 1x Assay buffer to the "Blank" wells.

3. Thaw **PCSK9(D374Y)** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube.

4. Dilute PCSK9(D374Y) in 1x Assay Buffer to 2.5 ng/µl (20 µl/well). Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

5. Prepare the test inhibitor/activator (referred as compound) to be tested (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 50 µl.

   a. If the compound is soluble in water, prepare a dilution of the compound in 1x Assay Buffer that is 10-fold higher than the final desired concentration. 1x Assay Buffer is the Diluent Solution.
   OR
   b. If the compound is soluble in DMSO, prepare it in 100% DMSO at a concentration 100-fold higher than the final desired concentration. Then dilute it 10-fold in 1x Assay Buffer to prepare the highest concentration of the 10-fold intermediate solutions. The concentration of DMSO is now 10%.

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

      For controls prepare 10% DMSO in 1x Assay Buffer (Diluent Solution) so that all wells contain the same amount of DMSO. The final concentration of DMSO should not exceed 1%.

6. Add 5 µl of compound dilution to each well designated "Test Compound".

7. Add 5 µl of Diluent Solution to the "Positive Control," "Uncoated Control" and "Blank" wells.

8. Add 20 µl of diluted PCSK9(D374Y) to all wells, except the "Blank".

9. Incubate at Room Temperature (RT) for two hours.

Component	Blank	Positive Control	Test Compound	Uncoated Control
1x Assay Buffer	45 µl	25 µl	25 µl	25 µl
Test Compound	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	5 µl
Diluted PCSK9(D374Y) (2.5 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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10. Remove the solution and wash the plate 3 times with 100 µl of 1x Assay buffer. Tap the plate onto clean paper towels to remove liquid.

11. Block by adding 100 µl of Blocking Buffer 2 to each well.

12. Incubate for 10 minutes at RT.

13. Remove the blocking buffer.

Step 3:

1. Dilute Streptavidin-HRP 1000-fold with Blocking Buffer 2.

2. Add 100 µl to each well.

3. Incubate for 1 hour at RT with gentle agitation.

4. Wash the plate three times with 1x Assay Buffer. Tap the plate onto clean paper towels to remove the liquid.

5. Block by adding 100 µl of Blocking Buffer 2 to each well.

6. Incubate for 10 minutes at RT.

7. Remove the Blocking Buffer and tap the plate onto clean paper towels to remove the liquid.

8. Just before use, mix on ice (100 µl/ well): N wells x (50 µl ELISA ECL substrate A + 50 µl ELISA ECL substrate B).

9. Add 100 µl to each well. Discard any unused chemiluminescent reagent after use.

10. Immediately read the plate in a luminometer or microtiter-plate capable of reading chemiluminescence.

11. The "Blank" value should be 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).

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Example Results

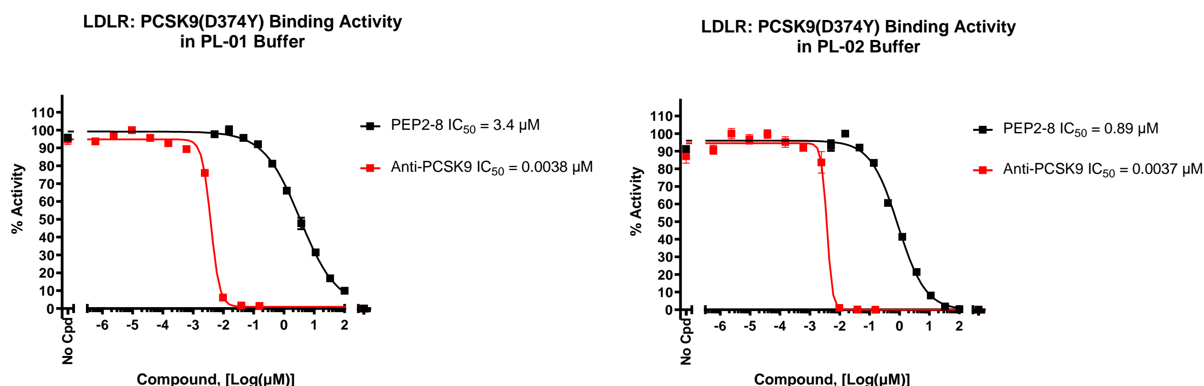

Figure 1. PCSK9-LDLR binding activity under acidic or neutral conditions.
Binding of PCSK9 to LDLR was measured using PL-01 (left) or PL-02 (right) buffers in the presence of increasing concentrations of Anti-PCSK9 Neutralizing Antibody (BPS Bioscience #71207) and PCSK9 inhibitor PEP2-8 (MedChemExpress #HY-P2276). Luminescence was measured using a Bio-Tek fluorescent microplate reader.

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

Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
PCSK9, C-terminal His-Avi-tag, Biotin-labeled	71305-1	25 µg
PCSK9(D374T)-LDLR TR-FRET Assay Kit	72011	384 reactions
PCSK9-LDLR TR-FRET Assay Kit	72010	384 reactions
Anti-PCSK9 Neutralizing Antibody	71207	50 µg
LDLR, FLAG-tag	71205	50 µg
LDLR, Biotin-labeled	71206-1	25 µg

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