Description

The PCSK9(D374T) [Biotinylated]-LDLR Binding Assay Kit is designed for screening and profiling purposes. The kit comes in a convenient 96-well format, with biotin-labeled mutant protein PCSK9(D374T), purified LDLR ectodomain, streptavidin labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled PCSK9(D374T) by streptavidin-HRP. Only a few steps on a microtiter plate are required for the assay. First, LDLR ectodomain is coated on a 96-well plate. Next, PCSK9(D374T) is incubated with LDLR on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can then be measured using a chemiluminescence reader.

Background

PCSK9 (Proprotein convertase subtilisin/kexin type 9) is known to function as a negative regulator of hepatic low-density lipoprotein receptors (LDLRs) by binding to the LDLR ectodomain. The D374T mutation is associated with 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 the LDLR.

Applications

Useful for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

Catalog #	Name	Amount	Storage
71211	PCSK9(D374T), Biotin-labeled*	10 µg	-80°C
71205	LDLR*	10 µg	-20°C
79742	Streptavidin-HRP	10 µl	-20°C
33298	3x PL-01 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

Name	Catalog #
PBS buffer	
Microplate reader capable of reading luminescence	
Adjustable micropipettor and sterile tips	

Stability

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

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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.

Assay Protocol

All samples and controls should be tested in duplicate.

Coating the plate with LDLR:

1. Thaw LDLR on ice. Upon first thaw, briefly spin tube containing LDLR to recover the full contents of the tube. Note: LDLR is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2. Dilute LDLR to 2 ng/µl in PBS.
3. Add 50 µl of diluted LDLR solution to each well and incubate overnight at 4°C. Leave a couple of wells empty (uncoated), for use with the "Ligand Control" (see below).
4. Dilute 3x PL-01 Assay buffer to 1x PL-01 Assay buffer with water.
5. Decant to remove supernatant. Wash the plate 3 times with 100 µl of 1x PL-01 Assay buffer. Tap the plate onto clean paper towels to remove liquid.
6. Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 1 hour at room temperature. Decant to remove supernatant.

Step 1:

7. Add 25 µl of 1x PL-01 Assay buffer prepared as directed in Step 4 to the "Positive control", "Test Inhibitor", and "Ligand Control". Add 45 µl of 1x PL-01 Assay buffer to the "Blank".
8. Thaw PCSK9(D374T) Biotin-labeled on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Note: PCSK9(D374T), Biotin-labeled is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.
9. Dilute PCSK9(D374T), Biotin-labeled in 1x PL-01 assay buffer at 2.5 ng/µl (each well will use 50 ng/20 µl). Keep diluted protein on ice until use. Discard any unused diluted protein after use.
10. Add 5 µl of inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control," "Ligand Control" and "Blank," add 5 µl of Diluent Solution (for example, 10% DMSO in water if the inhibitor was dissolved in DMSO).
11. Initiate the reaction by adding 20 µl of diluted PCSK9. Incubate at room temperature for two hours. Decant to remove supernatant. Wash the plate 3 times with 100 µl of 1x PL-01 Assay buffer. Tap the plate onto clean paper towels to remove liquid.
12. Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant.

Component	Blank	Positive Control	Test Inhibitor	Ligand Control
1x PL-01 assay buffer	45 µl	25 µl	25 µl	25 µl
Test inhibitor/Activator	-	-	5 µl	-
Diluent Solution	5 µl	5 µl	-	5 µl
PCSK9(D374T), [B] (2.5 ng/µl)	-	20 µl	20 µl	20 µl
Total	50 µl	50 µl	50 µl	50 µl

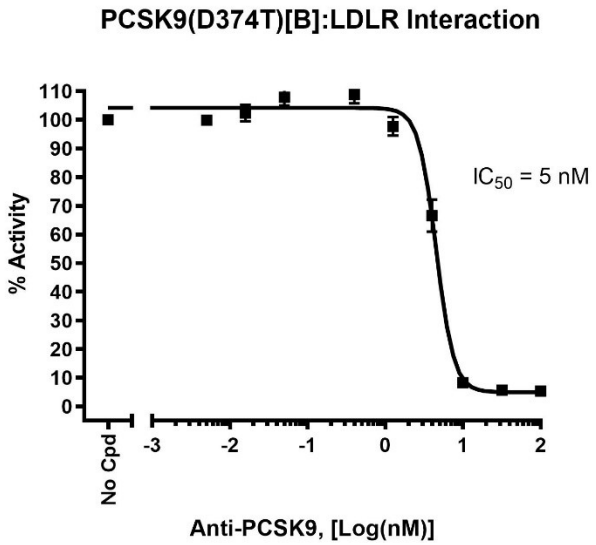
Step 2:

1. Dilute Streptavidin-HRP 1000-fold with Blocking Buffer 2.
2. Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.
3. Wash the plate three times with 1x PL-01 assay buffer. Tap the plate onto clean paper towels to remove liquid.
4. Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap the plate onto clean paper towels to remove liquid.
5. Just before use, mix on ice N wells x (50 µl ELISA ECL substrate A and 50 µl ELISA ECL substrate B), then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.
6. Immediately read the plate in a luminometer or microtiter-plate 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



PCSK9(D374T), Biotin-labeled-LDLR binding activity, measured using the PCSK9[Biotinylated]-LDLR Binding Assay Kit, BPS Bioscience, Catalog #78326 with Anti-PCSK9 antibody (#71207). 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

General considerations

"Blank" Control: The "Blank" control is important to determine the background absorbance in the assay.

Troubleshooting Guide

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

References

1. Chan, J.C. et al. (2009). *Proc. Natl Acad. Sci. USA*, **106,** 9820-9825.
2. Liang, H., et al. (2012) *J. Pharmacol. Exp. Ther*. **340,** 2289-236.

Related Products

Products	*Catalog #*	*Size*
PCSK9, His-tag	71204-1	20 µg
PCSK9, C-terminal His-Avi-tag, Biotin-labeled	71305-1	25 µg
PCSK9(D374T), Biotin-labeled	71211	25 µg
PCSK9(D374T)-LDLR TR-FRET Assay Kit	72011	384 rxns.
PCSK9-LDLR TR-FRET Assay Kit	72010	384 rxns.
Anti-PCSK9 Neutralizing Antibody	71207	50 µg
LDLR, FLAG-tag	71205	50 µg
LDLR, Biotin-labeled	71206-1	25 µg