Description

The PCSK9[Biotinylated]-LDLR Binding Assay Kit is designed for screening and profiling purposes. PCSK9 is known to function as a negative regulator of hepatic low-density lipoprotein receptors (LDLRs) by binding to the LDLR ectodomain. The PCSK9[Biotinylated]-LDLR Binding Assay Kit comes in a convenient 96-well format, with biotin-labeled PCSK9, 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 by streptavidin-HRP. Only a few steps are required for the assay. First, LDLR ectodomain is coated on a 96-well plate. Next, PCSK9 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, measured using a chemiluminescence reader.

**NOTE: As of November 2022, this protocol has been re-optimized for performance. Previous versions of this kit are available upon request.*

Background

PCSK9 regulates circulating cholesterol homeostasis by binding to the ectodomain of liver low-density lipid receptors and promoting their degradation. PCSK9 binds to the low density lipoprotein receptor (LDLR), the very low density lipoprotein receptor (VLDLR), the apolipoprotein E receptor (LRP1/APOER), and the apolipoprotein receptor 2 (LRP8/APOER2).

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage	
71304	PCSK9[Biotinylated]*	10 µg	-80°C	***Avoid multiple freeze/ thaw cycles***
71205	LDLR*	10 µg	-80°C	
79742	Streptavidin-HRP	10 µl	+4°C	
33298	3x PL-01 Assay Buffer	50 ml	-20°C	
79728	Blocking buffer 2	50 ml	+4°C	
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp	
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp	
79699	White 96-well microplate	1	Room Temp	

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

Materials Required but Not Supplied

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

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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[Biotinylated]-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) Thaw LDLR on ice. Briefly spin the tube containing LDLR to recover the full contents of the tube. Calculate the amount of protein required for the assay and dilute enough for the assay (100 ng/well).

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

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

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 the "Uncoated Control".

4) The following day, dilute 3x PL-01 Assay Buffer 3 times with water using 1 volume of stock PL-01 Assay Buffer and 2 volumes of distilled water.

5) Remove the liquid from the plate and wash the plate 3 times with 100 µl/well of 1x PL-01 Assay Buffer. Tap the plate onto clean paper towels to remove the liquid.

6) Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 1 hour at room temperature.

Step 1:

1) Prepare the Master Mix: N wells x (10 µl of 3x stock PL-01 Assay Buffer + 15 µl of distilled water)

2) Prepare the Test Inhibitor (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 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).

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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 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 Assay Buffer to keep the concentration of DMSO constant.

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

The final concentration of DMSO should not exceed 1%.

3) Remove the blocking buffer from the plate.

4) Add 25 µl of Master Mix to each well, including "Blank", "Uncoated control", "Positive Control" and "Test Inhibitor".

5) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control," "Uncoated Control" and "Blank," add 5 µl of 10% DMSO in 1x PL-01 Assay Buffer (Diluent Solution).

6) Add 20 µl of 1x PL-01 Assay Buffer to the well designated "Blank."

7) Thaw PCSK9[Biotinylated] on ice. Briefly spin the tube containing the protein to recover full the contents of the tube. Calculate the amount of protein required and dilute enough for the assay at a concentration of **2.5 ng/µl** (50 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.

8) Initiate the reaction by adding 20 µl/well of diluted PCSK9[Biotinylated]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	25 µl	25 µl	25 µl	25 µl
Test Inhibitor	–	–	–	5 µl
Diluent Solution (no Inhibitor)	5 µl	5 µl	5 µl	–
1x PL-01 Assay Buffer	20 µl	–	–	–
PCSK9[Biotinylated] (2.5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

9) Remove the liquid from the plate and wash the plate 3 times with 100 µl/well of 1x PL-01 Assay Buffer. Tap the plate onto clean paper towels to remove the liquid.

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10) Block wells by adding 100 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 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 the liquid.

4) Block wells by adding 100 µl of Blocking Buffer 2 to each well. Incubate for 10 minutes at room temperature. Remove the liquid from the plate. Tap the plate onto clean paper towels to remove the liquid.

5) Just before use, for each well, mix 50 µl of ELISA ECL substrate A and 50 µl of ELISA ECL substrate B, then add 100 µl of the mix to each well. Discard any unused chemiluminescent mix after use.

6) Immediately read 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).

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Example Results:

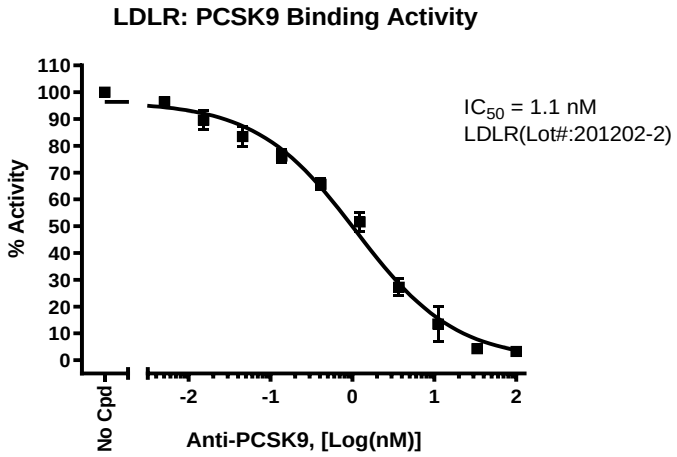

Figure 1: PCSK9-LDLR binding activity.
PCSK9-LDLR binding activity was measured using the PCSK9[Biotinylated]-LDLR Binding Assay Kit (BPS Bioscience #72002) in the presence of increasing concentrations of neutralizing Anti-PCSK9Antibody (BPS Bioscience #71207). Luminescence was measured using a Bio-Tek 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, His-Tag Recombinant	71204	50 µg
LDLR, FLAG-Tag Recombinant	71205	50 µg
PCSK9, C-terminal His-Avi-Tag, Biotin-Labeled Recombinant	71304	20 µg
LDLR, Biotin Labeled Recombinant	71206	20 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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