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Data Sheet

PCSK9[Biotinylated]-LDLR Binding Assay Kit

Catalog # 72002

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 simple steps on a microtiter plate 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, which can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71304	PCSK9, Biotinylated	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71205	LDLR	10 µg	-80°C	
79742	Streptavidin-HRP	15 µl	+4°C	
79727	3x PCSK9 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	White 96-well microplate	1	+4°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS buffer
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Rotating or rocker platform

APPLICATIONS: Great for screening small molecules and antibodies that inhibit the binding of PCSK9 to LDLR ectodomain.

STABILITY: One year from date of receipt when stored as directed.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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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.

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. Aliquot into single use aliquots. Immediately store remaining **LDLR** in aliquots at -80°C. *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 PCSK9 Assay buffer** to **1x PCSK9 Assay buffer** with water.

5) Decant to remove supernatant. Wash the plate 3 times with 100 µl **1x PCSK9 Assay buffer**. Tap 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:

1) Prepare the master mixture: N wells x (10 µl **3x PCSK9 assay buffer** + 15 µl H_2O)

2) Thaw **PCSK9-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **PCSK9-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. *Note: **PCSK9** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

3) Dilute **PCSK9-biotin** in **1x PCSK9 assay buffer** at 2.5 ng/µl (50 ng/20 µl). Keep diluted protein on ice until use. Discard any unused diluted protein after use.
4) Add 25 µl of master mixture to each well. Use uncoated wells for the "Ligand Control."

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	Blank	Ligand Control	Positive Control	Test Inhibitor
3x PCSK9 assay buffer	10 µl	10 µl	10 µl	10 µl
H_2O	15 µl	15 µl	15 µl	15 µl
Test Inhibitor/Activator	–	–	–	5 µl
10% DMSO in water (Inhibitor buffer)	5 µl	5 µl	5 µl	–
1x PCSK9 assay buffer	20 µl	–	–	–
PCSK9-biotin (2.5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control," "Ligand Control" and "Blank," add 5 µl of 10% DMSO in water (inhibitor buffer).

6) Add 20 µl of **1x PCSK9 Assay buffer** to the well designated "Blank."

7) Initiate reaction by adding 20 µl of diluted **PCSK9** (see Step 1-3). Incubate at room temperature for two hours.

8) Decant to remove supernatant. Wash the plate 3 times with 100 µl **1x PCSK9 Assay buffer**. Tap plate onto clean paper towels to remove liquid.

9) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant.

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 plate three times with **1x PCSK9 assay buffer**. Tap 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 plate onto clean paper towels to remove liquid.

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5) Just before use, mix on ice 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 sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “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 wave length 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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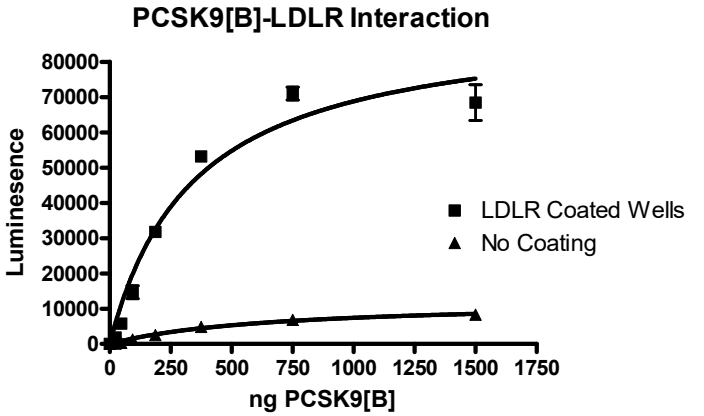
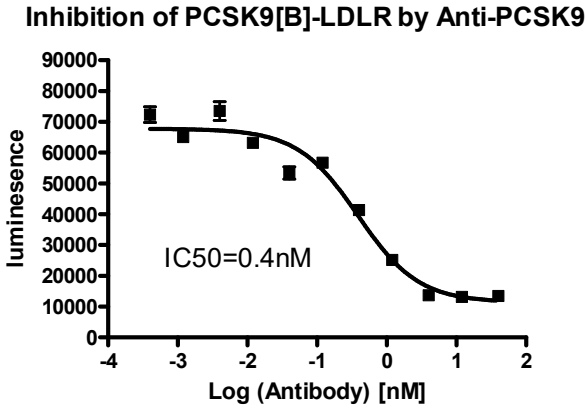
Example of Assay Results:





PCSK9-LDLR binding activity, measured using the using the PCSK9[Biotinylated]-LDLR Binding Assay Kit, BPS Bioscience, Catalog #72002 (both) and Anti-PCSK9 Inhibitor (right). Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product Name	Catalog #	Size
PCSK9	71204	50 µg
LDLR	71205	50 µg
PCSK9-biotin	71304	20 µg
LDLR-biotin	71206	20 µg
PCSK9 Neutralizing Ab	71207	50 µg
PCSK9-LDLR TR-FRET Assay Kit	72010	384 rxns.
PCSK9(D374T)-LDLR TR-FRET Assay Kit	72011	384 rxns.

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	PCSK9 or LDLR has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh PCSK9-biotin, (BPS Bioscience #71304) and fresh LDLR (BPS Bioscience #71205). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of PCSK9-biotin (BPS Bioscience #71304) to create a standard curve.