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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The PCSK9-LDLR Homogeneous Assay Kit is designed for screening and profiling purposes. The PCSK9-LDLR Homogeneous Assay Kit comes in a no-wash AlphaLISA® format, with sufficient PCSK9 and LDLR for 384 enzyme reactions. Two pre-formulated assay buffers are supplied with this kit to validate PCSK9-LDLR binding affinity in either neutral or acidic binding conditions. With this kit, two simple steps are required for binding measurement. First, PCSK9 enzyme is incubated with LDLR in the preferred buffer for one hour. Next, donor and acceptor beads are added, followed by reading the Alpha-counts.

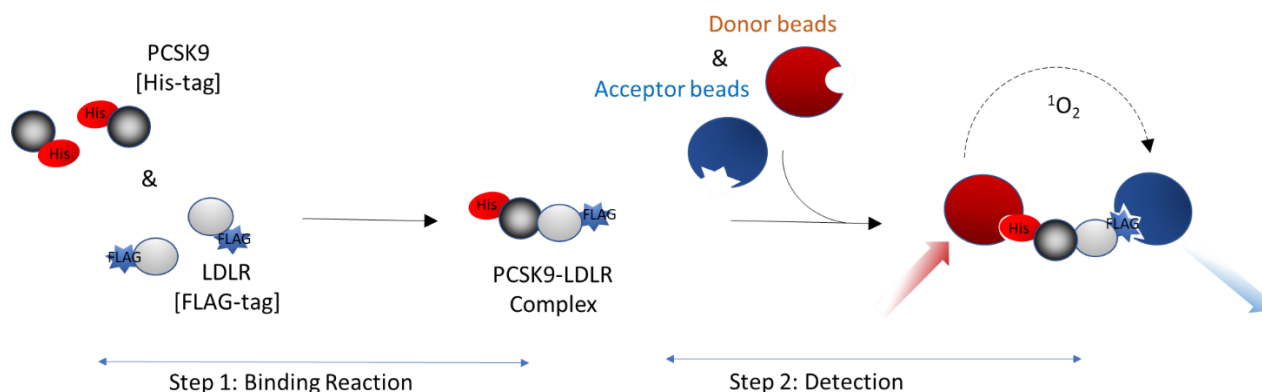


Figure 1: Illustration of the assay principle.

The FLAG-tag of LDLR binds to the anti-FLAG acceptor beads, while the His-tag on PCSK9 binds to Nickel Chelate donor beads, bringing the acceptor and donor beads in close proximity. Upon excitation of the donor bead a singlet oxygen is generated by the donor bead, which excites the acceptor bead and emits light proportionally to the level of interaction. AlphaLISA™ immunoassays are a no-wash alternative to ELISA immunoassays. These assays are robust, ideal for a minimal hands-on approach, and highly amenable to high-throughput applications.

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 molecule inhibitors in high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71204	PCSK9, His-Tag Recombinant*	2 x 10 µg	-80°C
71205	LDLR, FLAG-Tag Recombinant*	2 x 10 µg	-20°C
33298	3x PL-01 Assay Buffer (pH 6)	4 ml	-20°C
79727	3x PL-02 Assay Buffer (pH 7.4)	4 ml	-20°C

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

Materials Required but Not Supplied

- AlphaLISA® anti-FLAG acceptor beads, 5 mg/ml (PerkinElmer #AL112C)
- AlphaScreen® Nickel Chelate donor beads, 5 mg/ml (PerkinElmer #AS101D)
- Optiplate-384 (PerkinElmer #6007290)

Stability



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

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

Green and blue dyes that absorb light in the AlphaScreen® signal emission range (520–620 nm), such as Trypan Blue, interfere with the assay. Avoid using potent singlet oxygen quenchers such as sodium azide (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen® assays.

Assay Protocol

- All samples and controls should be performed in duplicates.
- The assay should include a “Negative control 1”, a “Negative control 2”, a “Positive control” and a “Test compound”.

Step 1:

1. Choose the neutral or acidic buffer at which you wish to test the interaction between PCSK9 and LDLR. **You can either run your reactions using acidic PL-01 Assay Buffer (pH 6.0) or neutral PL-02 Assay Buffer (pH 7.4).**
2. Thaw PCSK9, LDLR, and the neutral or acidic 3x Assay Buffer on ice. Briefly spin the tubes to recover the full contents. If the assay plate is going to be used more than once, prepare enough proteins for this portion of the assay and aliquot **the remaining undiluted proteins** into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.
3. 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.
4. Dilute LDLR to 4.2 ng/μl in 1x Assay Buffer. Keep the diluted protein on ice until use. Discard any unused diluted protein after use.
5. Dilute PCSK9 to 10.4 ng/μl in 1x Assay Buffer. Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

Note: The proteins are very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots more than once and do not re-use the diluted protein.

6. Prepare the Test Inhibitor (3 μ l/well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 15 μ l.
- a) If the Test Inhibitor is water-soluble, prepare serial dilutions of the inhibitor 5-fold more concentrated than the desired final concentrations using the 1x Assay Buffer. 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 a concentration 100-fold higher than the highest desired concentration in DMSO, then dilute the inhibitor 20-fold in 1x Assay Buffer to prepare the highest concentration of the 5-fold intermediate dilutions. The concentration of DMSO is now 5%.

Prepare serial dilutions of the Test Inhibitor at 5-fold the desired final concentrations using 5% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 5% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO in the reaction should not exceed 1%.

7. Add 6 μ l of diluted LDLR to all wells except “Negative control 1”.
8. Add 6 μ l of diluted PCSK9 to all wells except “Negative control 2”.
9. Add 3 μ l of Test Inhibitor dilutions to each well designated “Test Inhibitor.” For the “Positive Control,” “Negative Control 1” and “Negative control 2” add 3 μ l of Diluent Solution (for example, 5% DMSO in 1x Assay Buffer if the inhibitor was dissolved in DMSO).
10. Incubate at room temperature for one hour.

Component	Negative control 1	Negative control 2	Positive Control	Test Compound
1x Assay Buffer	6 μ l	6 μ l	-	-
Test inhibitor	-	-	-	3 μ l
Diluent Solution	3 μ l	3 μ l	3 μ l	-
PCSK9 (10.4 ng/ μ l)	6 μ l	-	6 μ l	6 μ l
LDLR (4.2 ng/ μ l)	-	6 μ l	6 μ l	6 μ l
Total	15 μ l	15 μ l	15 μ l	15 μ l



Protect your samples from direct exposure to light for step 2!

Step 2:

1. Dilute the anti-FLAG acceptor beads (PerkinElmer #AL112C) and the Nickel Chelate donor beads (PerkinElmer #AS101D) 500-fold with 1x Assay Buffer in one step. Add 10 μ L of acceptor/donor beads mixture per well.
2. Incubate 60-90 minutes at room temperature with slow shaking.
3. Read Alpha counts.

Example Results:

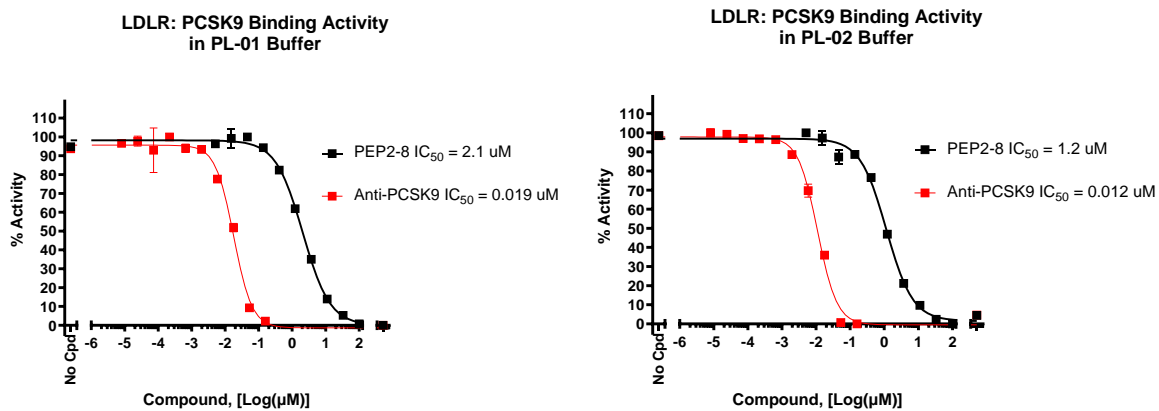


Figure 2. PCSK9-LDLR binding activity in neutral or acidic buffer.

Binding of PCSK9 to LDLR was measured using the PCSK9-LDLR Homogeneous Assay (BPS Bioscience #78812) using PL-01 or PL-02 buffer in the presence of increasing concentrations of Anti-PCSK9 Neutralizing Antibody (BPS Bioscience #71207) and PCSK9 inhibitor PEP2-8 (MedChemExpress #HY-P2276).

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.

Trademarks

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Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PCSK9, C-terminal His-Avi-tag, Biotin-labeled	71305	25 µg
PCSK9(D374T), Biotin-labeled	71211	25 µg
PCSK9(D374T)-LDLR TR-FRET Assay Kit	72011	384 reactions
PCSK9-LDLR TR-FRET Assay Kit	72010	384 reactions
LDLR, Biotin-labeled	71206	25 µg
PCSK9(His)-LDLR Binding Assay Kit	78813	2 sizes