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Data Sheet
PCSK9-LDLR TR-FRET Assay Kit
Catalog # 72010

DESCRIPTION:

The *PCSK9-LDLR TR-FRET Assay Kit* is designed to measure the inhibition of PCSK9 binding to LDLR in a homogeneous 384 reaction format. PCSK9 is known to function as a negative regulator of hepatic low-density lipoprotein receptors (LDLRs) by binding to the LDLR ectodomain. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; a sample containing europium-labeled (Eu) LDLR ectodomain, dye-labeled acceptor, biotin-labeled PCSK9, and an inhibitor is incubated for two hours. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
79079	LDLR-Eu	2 µg	-80°C	(Avoid freeze/ thaw cycles!)
71304	PCSK9, Biotinylated	30 µg	-80°C	
	Dye-Labeled Acceptor	2 x 10 µl	-20°C	
	3x PCSK9 TR-FRET Assay Buffer	4 ml	-20°C	
	White, Nonbinding, low volume, 384-well microtiter plate	1	Room temp.	

MATERIALS REQUIRED BUT NOT SUPPLIED:

Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: At least 6 months from date of receipt when stored as directed.

REFERENCE(S):

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.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

- 1) Dilute one part **3x PCSK9 TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x PCSK9 TR-FRET Assay Buffer**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C .
- 2) Dilute **Dye-Labeled Acceptor** 100-fold in **1x PCSK9 TR-FRET Assay Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C .
- 3) Thaw **LDLR-Eu** on ice. Upon first thaw, briefly spin tube containing **LDLR-Eu** to recover the full contents of the tube. Aliquot into single-use aliquots. Store remaining undiluted **LDLR-Eu** at -80°C immediately. *Note: **LDLR-Eu** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*
- 4) Dilute **LDLR-Eu** in **1x PCSK9 TR-FRET Assay Buffer** to 0.5 ng/ μl . Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C .
- 5) Prepare the master mixture: N wells \times (5 μl diluted **Dye-Labeled Acceptor** + 5 μl diluted **LDLR-Eu** + 3 μl **1x PCSK9 TR-FRET Assay Buffer**). Add 13 μl to every well.
- 6) Add 2 μl of inhibitor solution to each well designated "Test Inhibitor." Add 2 μl of 10% DMSO in water (inhibitor buffer) to the wells labeled "Negative Control" and "Positive Control."
- 7) Add 5 μl **1x PCSK9 TR-FRET Assay Buffer** to wells designated for "Negative Control."

	Positive Control	Negative Control	Test Inhibitor
Diluted Dye-Labeled Acceptor	5 μl	5 μl	5 μl
LDLR-Eu (0.5 ng/ μl)	5 μl	5 μl	5 μl
1x PCSK9 Assay Buffer	3 μl	8 μl	3 μl
Test Inhibitor	–	–	2 μl
10% DMSO in water (Inhibitor buffer)	2 μl	2 μl	–
PCSK9, biotinylated (8 ng/ μl)	5 μl	0 μl	5 μl
Total	20 μl	20 μl	20 μl

- 8) Thaw **PCSK9, Biotinylated** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **PCSK9, Biotinylated** into single-use aliquots. Store remaining undiluted **PCSK9, Biotinylated** in aliquots at -80°C

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immediately. Note: **PCSK9, Biotinylated** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.

- 9) Dilute **PCSK9, Biotinylated** in 1x **PCSK9 TR-FRET Assay Buffer** to 8 ng/ μ l. Initiate reaction by adding 5 μ l of diluted **PCSK9, Biotinylated** to wells designated for the "Positive Control" and "Test Inhibitor." Discard any remaining diluted PCSK9 protein after use.
- 10) Incubate at room temperature for 2 hours.
- 11) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	320 \pm 10 nm
Emission Wavelength	620 \pm 10 nm
Lag Time	60 μ s
Integration Time	500 μ s
Excitation Wavelength	320 \pm 10 nm
Emission Wavelength	665 \pm 10 nm
Lag Time	60 μ s
Integration Time	500 μ s

CALCULATING RESULTS:

Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

If desired, data can be normalized to percent inhibition. Typically for inhibitor screens the FRET value from the positive control is set to zero percent inhibition and the FRET value from the negative control is set to one hundred percent inhibition.

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EXAMPLE OF ASSAY RESULTS:

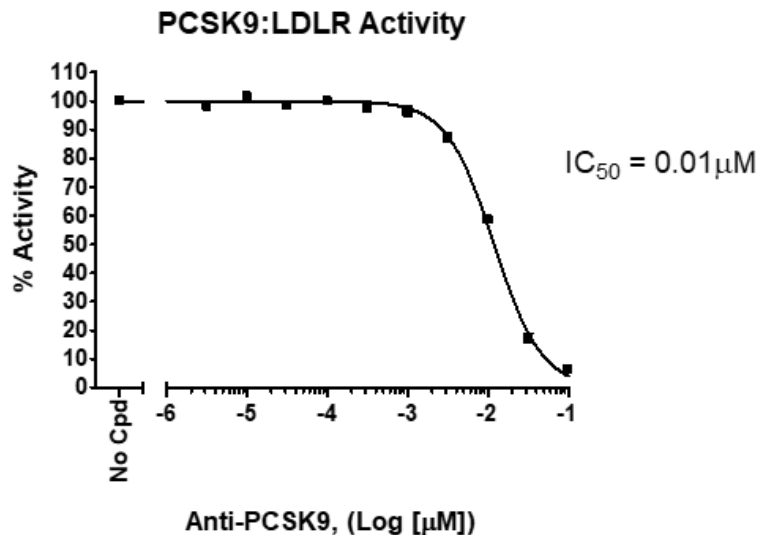


Figure Legend: Interaction of PCSK9 with LDLR. Data in the above graphs are expressed as FRET ratios. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

RELATED PRODUCTS:

<u>Product</u>	<u>Catalog #</u>	<u>Size</u>
PCSK9, His-tag	71204	50 µg
LDLR, FLAG-tag	71205	50 µg
PCSK9, C-terminal His-Avi, Biotin-labeled	71304	20 µg
LDLR, Biotin-labeled	71206	20 µg
PCSK9 Neutralizing Antibody	71207	50 µg
PCSK9[Biotinylated]-LDLR Binding Assay Kit	72002	96 rxns
PCSK9(D374T)-LDLR TR-FRET Assay Kit	72011	384 rxns

Note: The Dye-Labeled Acceptor used in this assay is a product of Cisbio Bioassays.

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