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<u>Data Sheet</u> PCSK9(D374T)-LDLR TR-FRET Assay Kit Catalog # 72011

DESCRIPTION:

The PCSK9(D374T)-LDLR TR-FRET Assay Kit is designed to measure the inhibition of the high affinity PCSK9 mutant (D374T) 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(D374T), and an inhibitor is incubated for two hours. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	LDLR-Eu	2 μg	-80℃	
71211	PCSK9(D374T), Biotinylated	25 μg	-80℃	Avoid
	Dye-labeled acceptor	2 x 10 μl	-20℃	(Avoid freeze/ thaw
	3x PCSK9 TR-FRET Assay Buffer	4 ml	-20℃	- cycles!)
	White, Nonbinding, low volume,	1	Room	
	384-well microtiter plate		temp.	

MATERIALS OR INSTRUMENTS 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.

Protocol for PCSK9(D374T) assay

- 1) Dilute one part **3x PCSK9 TR-FRET Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x PCSK9 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 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 Assay Buffer** to 0.5 μg/ml. 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 μ l diluted **Dye-labeled acceptor** + 5 μ l diluted **LDLR-Eu** + 3 μ l **1x PCSK9 Assay Buffer**). Add 13 μ l to every well.
- 6) Add 2 μl of inhibitor solution to each well designated "Test Inhibitor". Add 2 μl of the same solution without inhibitor (inhibitor buffer) to the wells labeled "Negative Control" and "Positive Control".
- 7) Add 5 µl 1x PCSK9 Assay Buffer to wells designated for "Negative Control."

	Positive Control	Negative Control	Test Inhibitor
Dye-labeled acceptor	5 μΙ	5 μΙ	5 μΙ
LDLR-Eu (0.5 μg/ml)	5 μΙ	5 μΙ	5 μΙ
1x PCSK9 Assay Buffer	3 μΙ	8 μΙ	3 μΙ
Test Inhibitor	_	_	2 μΙ
Inhibitor Buffer (no inhibitor)	2μΙ	2μΙ	_
PCSK9(D374T), biotinylated (8 μg/ml)	5 μΙ	0 μΙ	5 μΙ
Total	20 μΙ	20 μΙ	20 μΙ

8) Thaw **PCSK9(D374T)**, **biotinylated** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **PCSK9(D374T)**, **biotinylated** into single-use aliquots. Store remaining undiluted **PCSK9(D374T)**, OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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biotinylated in aliquots at -80°C immediately. *Note: PCSK9(D374T), biotinylated* is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.

- 9) Dilute PCSK9(D374T), biotinylated in 1x PCSK9 Assay Buffer to 8 μg/ml. Initiate reaction by adding 5 μl of diluted PCSK9(D374T), 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±10 nm		
Emission Wavelength	620±10 nm		
Lag Time	60 μs		
Integration Time	500 μs		
Excitation Wavelength	320±10 nm		
Emission Wavelength	665±10 nm		
Lag Time	60 μs		
Integration Time	500 μs		

CALCULATING RESULTS:

Two sequential measurements should be conducted. Eu-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).

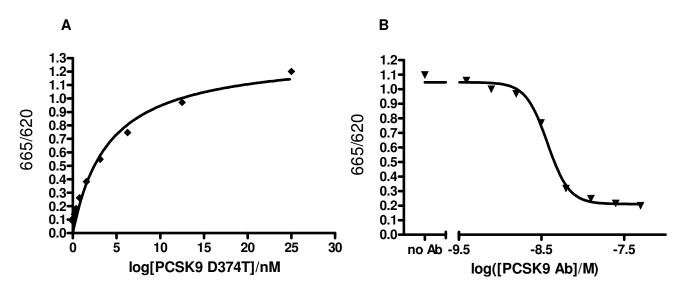
If desired, data can be normalized to percent inhibition. Typically for inhibitor screens the FRET value from the positive control is set to zero percent inhibition and the FRET value from the negative control is set to one hundred percent inhibition.



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EXAMPLE OF ASSAY RESULTS:



(A) Interaction of PCSK9(D374T) with LDLR-Eu. (B) Inhibition of PCSK9(D374T)/LDLR-Eu interaction using PCSK9 neutralizing antibody (PCSK9 Ab, Cat# 71207). 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:

Product Name	Catalog #	<u>Size</u>
PCSK9	71204	50 μg
LDLR	71205	50 μg
PCSK9-biotin	71304	20 μg
LDLR-biotin	71206	20 μg
PCSK9 neutralizing antibody	71207	50 μg
PCSK9[Biotinylated]-LDLR Binding Assay Kit	72002	96 wells
PCSK9-LDLR TR-FRET Assay Kit	72010	384 reactions

Note: The dye-labeled acceptor used in this assay is a product of Cisbio Bioassays.

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