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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



6044 Cornerstone Court W, Ste E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
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 °C	(Avoid freeze/ thaw cycles!)
71211	PCSK9(D374T), Biotinylated	25 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 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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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 $\mu\text{g/ml}$. 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 \pm 10 nm
Emission Wavelength	620 \pm 10 nm
Lag Time	60 μs
Integration Time	500 μs
Excitation Wavelength	320 \pm 10 nm
Emission Wavelength	665 \pm 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.

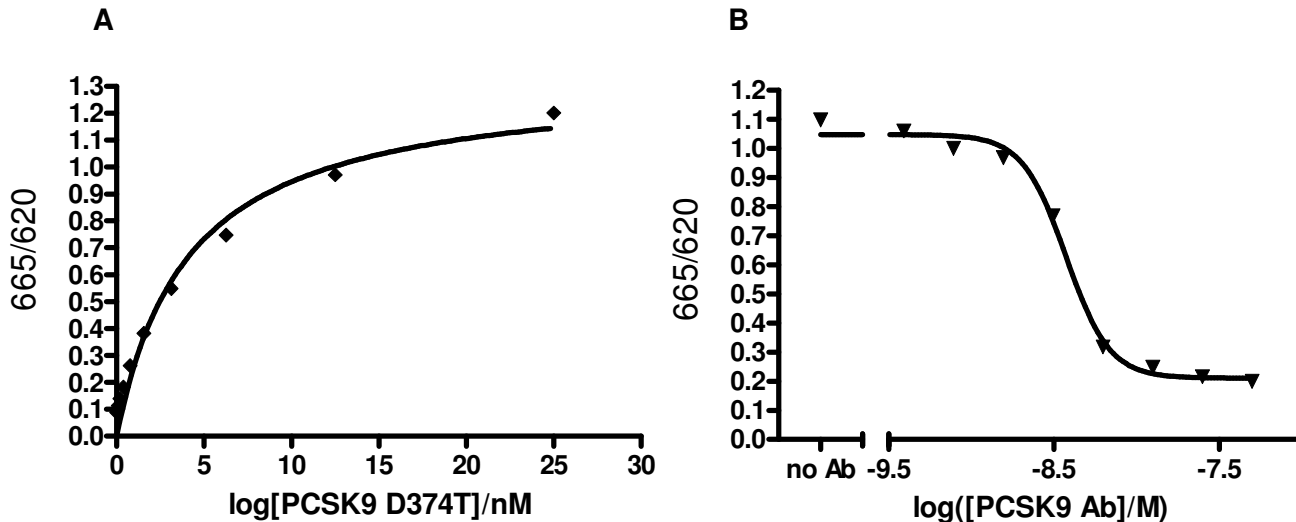
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EXAMPLE OF ASSAY RESULTS:



(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:

<u>Product Name</u>	<u>Catalog #</u>	<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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