

# **HUMAN PCSK-9 KITS**

### Part # 63ADK050PEG & 63ADK050PEH

Test size#: 500 tests (63ADK050PEG) and 10,000 tests (63ADK050PEH) - assay volume: 20 µL

Revision: #06 of March 2024

Store at: -60°C or below (63ADK050PEG); -60°C or below (63ADK050PEH)

For research use only. Not for use in diagnostic procedures.

### ASSAY PRINCIPLE

This kit is intended for the simple and rapid quantification of human Proprotein Convertase Subtilisin/Kexin type 9 (Human PCSK-9) in cell supernatants and offers a fast alternative to ELISA.

The detection principle of this kit is based on HTRF® technology (Homogeneous Time-Resolved Fluorescence). As shown in Figure 1, Human PCSK-9 is detected in a sandwich assay by using anti Human PCSK-9 antibody labeled with Europium cryptate (donor), and anti Human PCSK-9 antibody labeled with d2 (acceptor).

When the dyes are in close proximity, the excitation of the donor with a light source (laser or flash lamp) triggers a Fluorescence Resonance Energy Transfer (FRET) towards the acceptor, which in turn fluoresces at a specific wavelength (665 nm). Signal intensity is proportional to the number of antigen-antibody complexes formed and therefore to the Human PCSK-9 concentration.

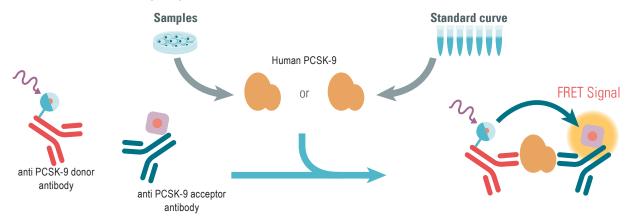


Figure 1: Principle of HTRF Human PCSK-9 sandwich assay.

### **MANUAL AT A GLANCE**

# ADD 10 µL Standard or Sample 5 µL anti-PCSK-9 acceptor antibody 5 µL anti-PCSK-9 donor antibody 7 pub antibody 2 hours @ RT 10 µL of pre-mixed Anti-Human PCSK-9 antibodies

Make sure to use the set-up for Eu Cryptate.

### **MATERIALS PROVIDED:**

KIT COMPONENTS	500 TESTS * CAT # 63ADK050PEG	10,000 TESTS * CAT # 63ADK050PEH
Human PCSK-9 Standard	1 vial - 10 μL	1 vial - 10 μL
Frozen	50 μg/mL	50 μg/mL
Human DCCK O Fu Cruntata Antihadu	1 vial - 50 μL	1 vial - 1 mL
Human PCSK-9 Eu Cryptate Antibody	Frozen - 50X	Frozen - 50X
Human DCCK 0 d2 Antihady	1 vial - 50 μL	1 vial - 1 mL
Human PCSK-9 d2 Antibody	Frozen - 50X	Frozen - 50X
Diluent **	1 vial	1 vial
ready-to-use	20 mL	20 mL
Detection buffer ***	1 vial	1 vial
	7 mL	105 mL
ready-to-use	Detection Buffer #3	Detection Buffer #3

<sup>\*</sup> When used as advised, the two available kit sizes will provide sufficient reagents for 500 tests and 10,000 tests respectively in 20 µL final volume. Assay volumes can be adjusted proportionally to run the assay in 96 or 1536 well microplates.

### **PURCHASE SEPARATELY:**

• HTRF®-Certified Reader. Make sure the setup for Eu Cryptate is used.

For a list of HTRF-compatible readers and set-up recommendations, please visit www.revvity.com

• Small volume (SV) detection microplates - .

For more information about microplate recommendations, please visit our website at: www.revvity.com

### STORAGE AND STABILITY

Store the kit at -60°C or below.

Under proper storage conditions, reagents are stable until the expiry date indicated on the label. Diluent and detection buffer are shipped frozen, but can be stored at 2-8°C in your premises.

If lyophilized, reconstituted reagents, antibodies, and standard stock solutions may be frozen and thawed only once. To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -16°C or below.

### REAGENT PREPARATION

### **BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- · Thaw the frozen reagents at room temperature, allow them to warm up to room temperature for at least 30 mins before use
- Before use, allow Diluent and Detection buffer to warm up at room temperature and homogenize them with a vortex.
- · Antibody solutions must be prepared in individual vials and can be mixed prior to dispensing.
- Human PCSK-9 standards (for standard curve) must be prepared in diluent or in the same medium as the samples.

TAKE CARE TO PREPARE STOCK AND WORKING SOLUTIONS ACCORDING TO THE DIRECTIONS FOR THE KIT SIZE YOU HAVE PURCHASED.

<sup>\*\*</sup> Marking like and military and lives and have a like and the distance of the second

<sup>\*\*</sup> Medium like cell culture medium can be an alternative to the diluent.

<sup>\*\*\*</sup> The Detection buffer is used to prepare working solutions of acceptor and donor reagents.

### TO PREPARE REAGENT STOCK SOLUTIONS:

500 TESTS KIT - 63ADK050PE	G	10,000 TESTS KIT - 63ADK050PEH		
	Anti-Human PCSK-9 E	Eu Cryptate antibody		
Thaw the Human PCSK-9 Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.	i	Thaw the Human PCSK-9 Eu Cryptate antibody. Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.		
	Anti-Human PCSK	K-9 d2 antibody		
Thaw the Human PCSK-9 d2 antibody . Mix gently. This 50X stock solution can be frozen and stored at -16°C or below.		Thaw the Human PCSK-9 d2 antibody . Mix ger This 50X stock solution can be frozen and stored -16°C or below.	,	
'	Human PCSK-	-9 Standard		
Thaw the Human PCSK-9 standard solution in order to obtain a 50 µg/mL stock solution. Mix gently.		Thaw the Human PCSK-9 standard solution in o obtain a 50 µg/mL stock solution. Mix gently.	der to	
	Diluer	nt		
The diluent is ready-to-use.		The diluent is ready-to-use.		
	Detection	buffer		
The Detection buffer is ready-to-use.		The Detection buffer is ready-to-use.		

### TO PREPARE ANTIBODY WORKING SOLUTIONS:

Each well requires 5  $\mu$ L of Human PCSK-9-Eu Cryptate Antibody and 5  $\mu$ L of Human PCSK-9-d2 Antibody.

Prepare the two antibody solutions in separate vials.

500 TESTS KIT - 63ADK050PEG		10,000 TESTS KIT - 63ADK050PEH		
	Human PCSK-9 Eu	Cryptate antibo	ody	
Dilute 50-fold the 50X stock solution (thawed reagent) of human PCSK-9 Eu Cryptate antibody stock solution with the Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.05 mL of Eu Cryptate antibody stock solution + 2.45 mL of Detection Buffer #3).	1 vol 49 vol	1 vol 4	9 vol	Dilute 50-fold the 50X stock solution (thawed reagent) of human PCSK-9 Eu Cryptate antibody stock solution with the Detection buffer #3: add 1 volume of Eu Cryptate antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 1 mL of Eu Cryptate antibody stock solution + 49 mL of Detection Buffer #3).
	Human PCSK	-9 d2 antibody		
Dilute 50-fold the 50X stock solution (thawed reagent) of human PCSK-9 d2 antibody stock solution with the Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 0.05 mL of d2 antibody stock solution + 2.45 mL of Detection Buffer #3).	1 vol 49 vol	1 vol	49 vol	Dilute 50-fold the 50X stock solution (thawed reagent) of human PCSK-9 d2 antibody stock solution with the Detection buffer #3: add 1 volume of d2 antibody stock solution in 49 volumes of Detection buffer #3 (e.g., 1 mL of d2 antibody stock solution + 49 mL of Detection Buffer #3).
	Antibo	dy mix		
It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).				It is possible to pre-mix the two ready-to-use antibody solutions just prior to dispensing the reagents by adding 1 volume of d2 antibody solution to 1 volume of Cryptate antibody solution (e.g. 1 mL of d2 antibody + 1 mL of Cryptate antibody).

### TO PREPARE STANDARD WORKING SOLUTIONS:

- Each well requires 10 μL of standard.
- Dilute the standard stock solution serially with diluent. Please note: If the sample to test is a cell supernatant, replace the diluent by culture medium.
- In order to check for a potential interference effect from your own assay buffer when using the assay for the first time, we highly recommend the parallel preparation of a standard curve in your own supplemented cell culture medium and in diluent.
- In order to counteract any standard sticking, we recommend changing tips between each dilution.

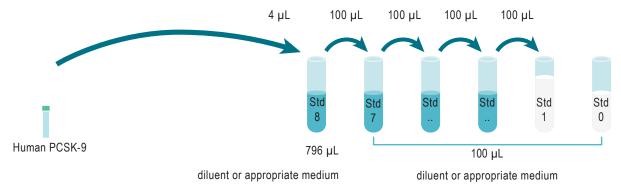
### A recommended standard dilution procedure is listed and illustrated below:

Dilute the standard stock solution 200-fold with diluent to prepare high standard (Std 8): e.g. take 4  $\mu$ L of standard stock solution and add it to 796  $\mu$ L of diluent. Mix gently.

Use the high standard (Std 8) to prepare the standard curve using 1/2 serial dilutions as follows:

- Dispense 100 µL of diluent in each vial from Std 7 to Std 0.
- Add 100  $\mu$ L of standard to 100  $\mu$ L of diluent, mix gently and repeat the 1/2 serial dilution to make standard solutions: std7, std6, std5, std4, std3, std2, std1.

This will create 8 standards for the analyte. Std 0 (Negative control) is diluent or appropriate culture medium alone.

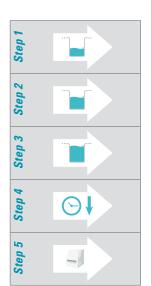


STANDARD	STANDARD SERIAL DILUTIONS	
Standard Stock solution	Thawed stock solution	50,000
Standard 8	4 μL Standard stock solution + 796 μL Diluent	250
Standard 7	100 μL standard 8 + 100 μL Diluent	125
Standard 6	100 μL standard 7 + 100 μL Diluent	62.5
Standard 5	100 μL standard 6 + 100 μL Diluent	31.3
Standard 4	100 μL standard 5 + 100 μL Diluent	15.6
Standard 3	100 μL standard 4 + 100 μL Diluent	7.8
Standard 2	100 μL standard 3 + 100 μL Diluent	3.9
Standard 1	100 μL standard 2 + 100 μL Diluent	1.95
Standard 0	100 μL Diluent	0

### **TO PREPARE SAMPLES:**

- Each well requires 10 μL of sample.
- Just after their collection, put the samples at 4°C and test them immediately. For later use, samples should be dispensed into disposable plastic vials and stored at -60°C or below. Avoid multiple freeze/thaw cycles.
- Samples with a concentration above the highest standard (Std 8) must be diluted diluent

# ASSAY MANUAL



Standard (Std 0 - Std 8) Samples				
Dispense 10 µL of each Human PCSK-9 standard (Std 0 - Std 8) into each standard well				
Add 5 µL of Human PCSK-9 d2 antibody working solution to all wells				
Add 5 μL of Human PCSK-9 Eu Crypta	te antibody working solution to all wells			
Seal the plate and incubate 2 hours @ RT				
Remove the plate sealer and read on an HTRF® compatible reader				

5 μL Human PCSK-9-Eu Cryptate         5 μL Human PCSK-9-Eu Cryptate         5 μL Human PCSK-9-Eu Cryptate         6 μL Human PCSK-9-Eu Cryptate         7 μL Human PCSK-9-Eu Cryptate         7 μL Human PCSK-9-Eu Cryptate         7 μL Human PCSK-9-Eu Cryptate         8 Repeat Well B1         8 Repeat Well B2         7 μL Human PCSK-9-42         8 Repeat Well B4	5 μL Human PCSK-9-d2 5 μL Human PCSK-9-Eu Cryptate         Repeat Well A1         Repeat Well A1         5 μL Human PCSK-9-d2 5 μL Human PCSK-9-Eu Cryptate         Repeat Well A4         Repeat Well B4         Repeat Well C4         Repeat Well C4 <t< th=""><th></th><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th></t<>		1	2	3	4	5	6
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5 µL Human PCSK-9-Eu Cryptate		5 µL Hu		Repeat Well H1	Repeat Well H1	5 µL		
						K L M		
H						0 P		

## DATA REDUCTION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

Ratio = 
$$\frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

3. Calculate the % delta F which reflects the signal to background of the assay. The negative control (Standard 0) plays the role of an internal assay control. Delta F is used for the comparison of day to day runs of the same assay.

For more information about data reduction, please visit www.revvity.com

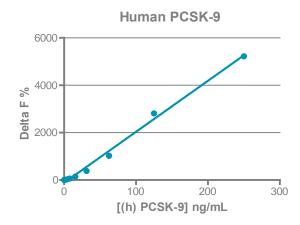
### **RESULTS**

This data must not be substituted for the data obtained in the laboratory and should be considered only as an example.

Results may vary from one HTRF® compatible reader to another.

The assay standard curve is created by plotting delta F% versus the analyte concentration:

	Ratio (1)	CV (2)	Delta F% (3)
Standard 0 - Negative control	707	3%	0%
Standard 1 - 1.95 ng/mL	807	1%	14%
Standard 2 - 3.9 ng/mL	896	1%	27%
Standard 3 - 7.8 ng/mL	1,141	2%	61%
Standard 4 - 15.6 ng/mL	1,701	3%	141%
Standard 5 - 31.3 ng/mL	3,415	1%	383%
Standard 6 - 62.5 ng/mL	7,923	8%	1,021%
Standard 7 - 125 ng/mL	20,585	6%	2,813%
Standard 8 - 250 ng/mL	37,606	4%	5,222%



# ANALYTICAL CHARACTERISTICS Sensitivity: 2 ng/mL (in diluent)

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