

# Protocol for APC-PCI Matched Reagent Set™ (For Research Use Only)

#### BACKGROUND

Activated protein C - protein C inhibitor (APC-PCI) complex is formed in the blood circulation upon activation of protein C (PC) and is thus a marker of PC activation. Protein C is a serine protease zymogen synthesized by the liver. The mature protein is glycosylated and has a molecular mass of approximately 62 kDa.

## INTRODUCTION APC-PCI MATCHED REAGENT SET™

APC-PCI Matched Reagent Set<sup>™</sup> enables you to measure the complex between Activated Protein C and Protein C inhibitor. These reagents are designed to be used in a classical sandwich ELISA setup and the detailed protocol description of the assay is described below.

## OVERVIEW OF CRITICAL REAGENTS

Reagent name	Product code	Component size
APC-PCI Complex specific antibody	JST 001-38-02	200 μg (1 mg/mL)
APC-PCI Biotinylated detection antibody	SA001RA	50 μg (1 mg/mL)
APC-PCI Calibrator	SP007RA	1 mL (1 ng/mL) -lyophilized
APC-PCI Sample Diluent	SB001RA	50 mL
APC-PCI Matched Reagent Set Protocol	YM001RX	-

#### ASSAY PROCEDURE

- Dilute the APC-PCI complex specific antibody (JST 001-38-02) in PBS (0.14 M NaCl, 0.01M phosphate, 0.0027M potassium chloride, pH 7.4) to a concentration of 5 μg/mL.
- 100 µL of the above solution is dispensed into each well of an ELISA plate (e.g. 96 well MaxiSorp<sup>™</sup>, (Nunc) and the plate is incubated overnight at 2-8°C in humid conditions.
- Preparation of calibrators: Take 1 vial of lyophilized calibrator material (SP007RA) and reconstitute the vial by adding 1 mL ELGA H<sub>2</sub>O to the vial. This constitutes the 1000 pg/mL stock. Reconstituted freezedried calibrator stock can stand up to 5 thaw/freeze cycles when frozen at -20°C).
- Make up calibrators 1 to 8 by diluting the reconstituted calibrator in Sample Diluent (SB001RA) according to the following scheme.

	Volume 1000 pg/mL stock (μL)	Volume Sample Diluent (μL)	Final volume (µL)	Calibrator value (pg/mL)
Reconstituted lyophilized vial	1000	-	787*	1000
Calibrator 8	100	213	313	320
Calibrator 7	50	263	313	160
Calibrator 6	25	288	313	80
Calibrator 5	16	297	313	50
Calibrator 4	10	303	313	30
Calibrator 3	6	384	390	16
Calibrator 2	6	775	781	8
Calibrator 1	-	500	500	0

\*Aliquot and re-freeze if not all plates are used.

BioPorto Diagnostics A/S • Tuborg Havnevej 15, st. • DK-2900 Hellerup • Denmark • info@bioporto.com www.bioporto.com • Phone: +45 4529 0000 • Fax: +45 4529 0001 • CVR/VAT: DK-1864 5882



		·``	<ul> <li>Wash<sup>1</sup> plate 3 times.</li> </ul>
	ዠ APC-PCI antibody	1	• Pipette 90 $\mu\text{L}$ volumes of Sample Diluent (SB001RA) into each
	Coat the plates with the APC- PCI antibody (JST 001-38)		well designated for sample and add 10 $\mu\text{L}$ sample.
		1 hour	$\bullet$ Transfer 100 $\mu L$ calibrator to each well in double
	APC-PCI complex		determinations.
° o tute	Diluted samples and reconsti- tuted calibrators are added to each well and incubated		• Incubate 1 hour at room temperature on a shaking platform.
		+	Wash plate 3 times.
代	片 <sup>®</sup> Biotinylated PC antibody	1 hour	• Dilute APC-PCI Biotinylated detection antibody (SA001RA) to
			a concentration of 0.26 $\mu\text{g}/\text{mL}$ (e.g. wash buffer with 0.5%
A second se Second second s	Biotinylated detection antibo- dy is added to each well and		BSA).
		+ 1	$\bullet$ Dispense 100 $\mu L$ of diluted APC-PCI Biotinylated detection
✓ HRP-Streptavidin HRP-conjugated streptavidir is added to each well and in- cubated Image: Streptavidir	1 hour	Antibody (SA001RA) into each well.	
			<ul> <li>Incubate 1 hour at room temperature on a shaking platform.</li> </ul>
	is added to each well and in-		• Wash plate 3 times.
	cubated	10 min.	<ul> <li>Dispense 100 μL HRP-Streptavidin<sup>2</sup></li> </ul>
	IMB Substrate	0	<ul> <li>Incubate 1 hour at room temperature on a shaking platform.</li> </ul>
म्ल्रेम् we	Substrate is added to each well. Develop for 10 minutes in the dark		• Wash plate 3 times.
		1	<ul> <li>Dispense 100 μL TMB substrate<sup>3</sup>.</li> </ul>
	Stop solution		<ul> <li>Incubate 10 min in the dark.</li> </ul>
Stop solution is added to each			• Add 100 $\mu L$ 0.5 M $H_2SO_4$ to each well.
	well. Quantitative results are ob- tained by measuring the absor-		<ul> <li>Read the absorbance of the wells at 450 nm and calculate</li> </ul>
bances of the wells at 450 nm	V I	APC-PCI content in complex (remember to multiply by dilution	

APC-PCI content in samples (remember to multiply by dilution

factor) from calibration curve.

<sup>&</sup>lt;sup>1</sup> E.g. Wash buffer: Trizma base (10 mmol/L), NaCl (140 mmol/L), Tween 20 (0.05 % (v/v)), pH 7.4.

 $<sup>^2</sup>$  Use a suitable HRP-Streptavidin reagent. We recommend HRP-Streptavidin from Pierce, (cat no: 21140), 6  $\mu$ g/mL.

<sup>&</sup>lt;sup>3</sup> Use a suitable TMB. We recommend TMB E-tra, Ready-to-use Substrate, from KemEnTec A/S (cat. no. 4800).