

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™ 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

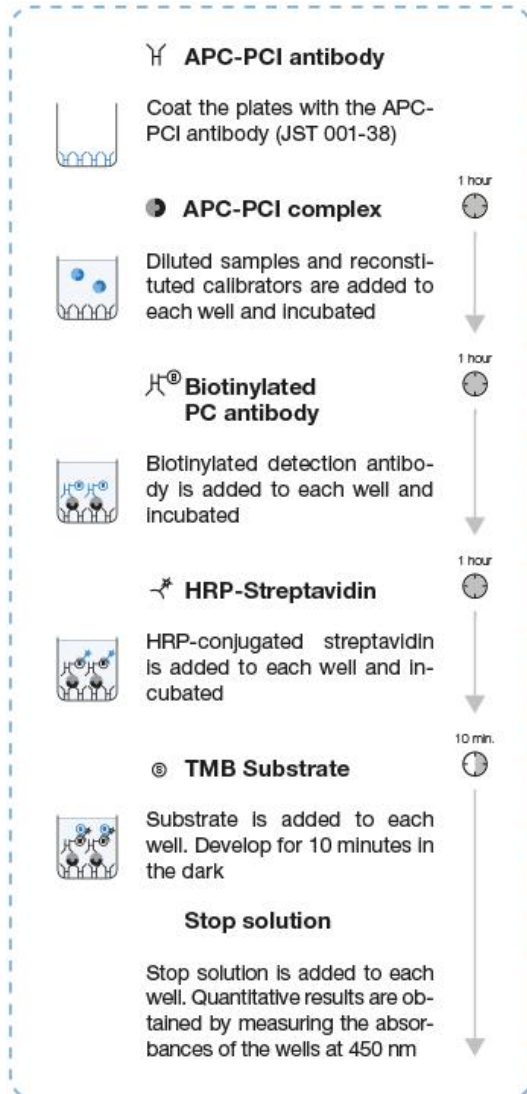
Reagent name	Product code	Component size
APC-PCI Complex specific antibody	JST 001-38-02	200 µg (1 mg/mL)
APC-PCI Biotinylated detection antibody	SA001RA	50 µg (1 mg/mL)
APC-PCI Calibrator	SP007RA	1 mL (1 ng/mL) -lyophilized
APC-PCI Sample Diluent	SB001RA	50 mL
APC-PCI Matched Reagent Set Protocol	YM001RX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

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™, (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 H₂O to the vial. This constitutes the 1000 pg/mL stock. Reconstituted freeze-dried 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.



- Wash¹ plate 3 times.
- Pipette 90 μ L volumes of Sample Diluent (SB001RA) into each well designated for sample and add 10 μ L sample.
- Transfer 100 μ L calibrator to each well in double determinations.
- Incubate 1 hour at room temperature on a shaking platform.
- Wash plate 3 times.
- Dilute APC-PCI Biotinylated detection antibody (SA001RA) to a concentration of 0.26 μ g/mL (e.g. wash buffer with 0.5% BSA).
- Dispense 100 μ L of diluted APC-PCI Biotinylated detection Antibody (SA001RA) into each well.
- Incubate 1 hour at room temperature on a shaking platform.
- Wash plate 3 times.
- Dispense 100 μ L HRP-Streptavidin²
- Incubate 1 hour at room temperature on a shaking platform.
- Wash plate 3 times.
- Dispense 100 μ L TMB substrate³.
- Incubate 10 min in the dark.
- Add 100 μ L 0.5 M H₂SO₄ to each well.
- Read the absorbance of the wells at 450 nm and calculate APC-PCI content in samples (remember to multiply by dilution factor) from calibration curve.

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

² Use a suitable HRP-Streptavidin reagent. We recommend HRP-Streptavidin from Pierce, (cat no: 21140), 6 μ g/mL.

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