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

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

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.

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

*Aliquot and re-freeze if not all plates are used.
Wash plate 3 times.

Pipette 90 μL volumes of Sample Diluent (SB001RA) into each well designated for sample and add 10 μL sample.

Transfer 100 μL 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 μg/mL (e.g. wash buffer with 0.5% BSA).

Dispense 100 μL 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 μL HRP-Streptavidin.

Incubate 1 hour at room temperature on a shaking platform.

Wash plate 3 times.

Dispense 100 μL TMB substrate.

Incubate 10 min in the dark.

Add 100 μL 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.

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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), diluted to 50 ng/mL in SB001RA. The dilution should be made fresh before use.

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

**Mouse monoclonal antibody**

**JST 001-38**

<table>
<thead>
<tr>
<th>CAT. NO.</th>
<th>Subclass: IgG1/k</th>
<th>Clone: M36</th>
</tr>
</thead>
</table>

**SPECIFICITY**

JST 001-38 is specific for APC-PCI complex and cleaved PCI but has little reactivity towards native PCI. The dissociation constants (as determined by standard surface plasmon resonance technique) are: Kd = 4x10e-10 M for PCI in complex with APC and 2x10e-10 M for cleaved PCI. The value of Kd for native PCI is too low to measure, i.e. > 10e-5 M.

**IMMUNOGEN**

Mixture of PCI in complex with PSA (approx. 80%) and PCI cleaved from such a complex (approx. 20%).

**TESTED APPLICATIONS**

ELISA, WB (Not applicable)

**SPECIES REACTIVITY**

Positive: 
- Human

**SPECIES REACTIVITY**

Negative: 
- Not determined

**EPITOPE SPECIFICITY**

The epitope is located in the calcium-binding N-terminal EGF domain of protein C.

**PRESENTATION**

Content: Available in 200 µL and 1 mL size. 1 mg/mL ±/− 15%. See Certificate of Analysis for details.

Preparation: Protein-A purified

Form: Liquid

Solvent:
- 0.01 M phosphate buffer, pH 7.4, with 0.14 M NaCl and 15 mM sodium azide

Storage: 4-8°C without exposure to light. No precautions necessary during handling.

**APPLICATION**

**ELISA:** JST 001-38 shows excellent reactivity towards PCI in cleaved and complex form in ELISA. JST 001-38 is a part of the APC-PCI Matched Reagent Set, and can be used in ELISA as a capture antibody in combination with SA001RA as the detection antibody (1-5).

**WB:** JST 001-38 shows low reactivity against denatured antigen in SDS-PAGE.

**TARGET**

Activated Protein C (APC), a serine proteinase is inhibited by Protein C inhibitor (PCI). PCI belongs to a group of inhibitors sometimes referred to as serpines (serine proteinase inhibitors), and form 1:1 complexes with APC. PCI plasma concentration is approximately 4 ug/ml. The complex formation between APC and PCI proceeds at a slow rate, which is manifested by a long half-life for APC in plasma, wherein t½ is about 20 min. However, the rate of complex formation between APC and PCI is increased by heparin. Upon complex formation with APC, the serpin is cleaved in its so-called bait region, whereby a stable intermediate acyl complex is formed. With time, the intermediate acyl complex dissociates, whereby APC is regenerated and a proteolytically modified, i.e. cleaved, inactive serine proteinase inhibitor is formed.

**REFERENCES**


**CONDITIONS**

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APC-PCI Biotinylated detection antibody  
Mouse monoclonal antibody, biotinylated  
SA001RA

**SPECIFICITY**  
SA002RA binds to human Activated Protein C - Protein C Inhibitor (APC-PCI) complex.

**IMMUNOGEN**  
Protein C

**TESTED APPLICATIONS**  
ELISA

**SPECIES REACTIVITY**  
Human

**SPECIES REACTIVITY**  
Not determined

**EPITOPE SPECIFICITY**  
Not determined

**PRESENTATION**  
Content: 50 µL, 1 mg/mL +/- 15%. See Certificate of Analysis for details.
Preparation: Biotinylated
Form: Liquid
Solvent: 0.01 M phosphate buffer, pH 7.4, with 0.14 M NaCl and 15 mM sodium azide
Storage: 4-8ºC without exposure to light. No precautions necessary during handling.

**APPLICATION**  
SA001RA is a part of the APC-PCI Matched Reagent Set, and can be used in ELISA as a detection antibody in combination with JST 001-28 as the capture antibody.

**TARGET**  
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. Its concentration in plasma is 3-5 µg/mL and its half-life in the circulation 6-8 hours. PC is slowly activated by thrombin and 1000-fold more rapidly activated by thrombin in complex with thrombomodulin on the vascular surface of endothelial cells. Here the activation of PC is further 20-fold enhanced by the binding of PC to the endothelial PC receptor (EPCR).

**REFERENCES**

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<table>
<thead>
<tr>
<th>PRODUCT NAME</th>
<th>APC-PCI Calibrator Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRODUCT NO.</td>
<td>SP007RA</td>
</tr>
<tr>
<td>PRESENTATION</td>
<td>Content: 1 mL, 1ng/mL</td>
</tr>
<tr>
<td></td>
<td>Solvent: 8 mM sodiumphosphate and 2 mM potassium phosphate buffer with 0.2 M NaCl, stabilizing proteins and preservatives, pH 7.4.</td>
</tr>
<tr>
<td></td>
<td>Storage: Store the vial of freeze-dried material at -20°C</td>
</tr>
<tr>
<td></td>
<td>Stability: Use before expiry on label. Reconstituted freeze-dried calibrator stock remains stable for 24 h at 2-8°C. Reconstituted freeze-dried calibrator stock can stand up to 5 thaw/freeze cycles (freeze at -20°C)</td>
</tr>
</tbody>
</table>

APPLICATION  
SP007RA is a part of the APC-PCI Matched Reagent Set, and can be used in ELISA together with JST 001-38 as the capture antibody and SA001RA as the biotinylated detection antibody.

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. Its concentration in plasma is 3-5 µg/mL and its half-life in the circulation 6-8 hours. PC is slowly activated by thrombin and 1000-fold more rapidly activated by thrombin in complex with thrombomodulin on the vascular surface of endothelial cells. Here the activation of PC is further 20-fold enhanced by the binding of PC to the endothelial PC receptor (EPCR).
**PRODUCT NAME**  
APC-PCI Sample Diluent

**PRODUCT NO.**  
SB001RA

**PRESENTATION**  
Content: 50 mL  
Solvent: Buffer with salt, proteins and preservatives.  
Storage: 2-8°C  
Stability: See label

**APPLICATION**  
SB001RA is a part of the APC-PCI Matched Reagent Set, and should be used to dilute the samples and calibrator when performing the ELISA.

**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. Its concentration in plasma is 3-5 µg/mL and its half-life in the circulation 6-8 hours. PC is slowly activated by thrombin and 1000-fold more rapidly activated by thrombin in complex with thrombomodulin on the vascular surface of endothelial cells. Here the activation of PC is further 20-fold enhanced by the binding of PC to the endothelial PC receptor (EPCR).

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APC-PCI COMPLEX SPECIFIC – monoclonal antibody

MATCHED REAGENT SET

- Based on a unique APC-PCI Complex specific antibody
- Highly specific outcome of your ELISA sandwich set-up
- Market exclusive Matched Reagent Set for use in your research

APC-PCI Complex:
A pioneering role in sepsis research

Sepsis is characterized by systemic activation of the inflammatory and coagulation cascades in response to microbial infection. The role APC-PCI plays in sepsis and in sepsis management is still being discovered. Some studies indicate that APC-PCI levels in patients with severe sepsis may have an impact on their outcome (61).

APC-PCI Complex:
A key role in coagulation research

Activated Protein C- Protein C Inhibitor (APC-PCI) complex is formed upon activation of Protein C (PC) into APC, which in turn is inactivated immediately by PCI, making APC-PCI a marker of PC activation. APC-PCI is a part of the coagulation cascade where it plays an important role as an anticoagulant, by cleaving several blood coagulation factors and limiting blood coagulation. APC-PCI levels are elevated in several thrombotic conditions, and measuring APC-PCI levels has been shown to be relevant in several conditions such as deep vein thrombosis, myocardial infarctions and aortic aneurysms (1-5).
APC-PCI COMPLEX SPECIFIC – monoclonal antibody
MATCHED REAGENT SET

For Research Use Only. Not for use in diagnostic procedures.

References


