APC-PCI ELISA Kit

KIT 040

BioPorto®
Diagnostics
Please read these instructions carefully

APPLICATION
For the measurement of activated protein C - protein C inhibitor complex in human plasma.
For research use only. Not for use in diagnostic procedures.

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 (PC) 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).

Activated protein C (APC) splits blood coagulation factors Va and VIIIa, using protein S and blood coagulation factor V as cofactors. It thereby limits blood coagulation in the vicinity of membrane-bound factors Va and VIIIa. APC may also have an indirect fibrinolytic activity by inhibiting plasminogen activator inhibitor-1 and by limiting the generation of activated thrombin-activatable fibrinolysis inhibitor. APC may also have an anti-inflammatory action by inhibiting the production of tumor necrosis factor by monocytes, by blocking the adhesion of leukocytes to selectins, and by limiting thrombin-induced endothelial inflammatory responses. In addition, it has been demonstrated to have an inhibitory effect on apoptosis.

APC has a molecular mass of approximately 55 kDa and its normal concentration in plasma has been estimated as lying within the range of 1-3 ng/mL. The half-life of APC in the human circulation is short, being variously estimated as 20 minutes1 or 45 minutes2.

As soon as APC is produced within the circulation, it undergoes inactivation by reacting with serpins (serine protease inhibitors) which are also present in the blood. These include protein C inhibitor (PCI), α1-antitrypsin, α2-antiplasmin and C1-esterase inhibitor. Of these, PCI appears to be of the greatest physiological importance. PCI is a single-chain protein with a molecular mass of approximately 57 kDa and its normal concentration in plasma is about 5 µg/mL. Its half-life in the circulation, measured in rabbits, is about 24 hours. APC-PCI complex is formed in vitro with a second order rate constant of 1.3 x 104 M^{-1}s^{-1}. This is 1000 fold faster than complex formation with α1-antitrypsin. The half-life of APC-PCI in the circulation is short, about 20 minutes in rabbits, 40 minutes in baboons.

The median concentration of APC-PCI complex measured in plasma from healthy volunteers was 0.13 ng/mL (range 0.07-0.26 ng/mL), blood samples being collected into acid citrate to prevent further complex formation after sampling.

APC-PCI in research
Sepsis: Sepsis is a devastating disorder characterized by systemic activation of the inflammatory and coagulation cascades in response to microbial infection. The mortality of severe sepsis is approximately 30%. Apart from antimicrobial therapy, the infusion of human recombinant APC (Xigris®, Eli Lilly) is the only treatment documented to reduce the mortality of sepsis patients. The effect is modest; however, with an absolute reduction in mortality of 6.1%. Much effort is being invested in finding a way of predicting which patients with sepsis are most likely to benefit from this type of treatment.

In a preliminary study of APC-PCI levels in sepsis patients conducted by BioPorto Diagnostics, a wide variation in the activation of the PC pathway was found. Patients with a high degree of PC activation (APC-PCI >0.72 ng/mL) or no activation (APC-PCI <0.25 ng/mL) showed an elevated mortality (44-50%), while patients with moderate PC activation (APC-PCI 0.26-0.72 ng/mL) showed a low mortality (13%). This suggests that APC-PCI measurement may find a role in predicting outcome and guiding treatment with APC.
**Thrombosis:** Plasma APC-PCI levels are elevated in several thrombotic conditions. In a case control study of patients suspected of deep vein thrombosis, APC-PCI levels discriminated cases from controls as efficiently as levels of D-dimer\(^{10}\), the recognized biomarker for this condition. APC-PCI levels are raised after myocardial infarction\(^{11}\) and aortic aneurysm\(^{12}\) and have been found to be predictive of outcome after aortic surgery\(^{13}\).

**SAMPLE MATERIAL**

To obtain results that reflect the circulating level of APC-PCI, measurements should be made in plasma prepared from blood samples collected into acid citrate (e.g. into 5-mL vacuum tubes containing 0.5 mL of 0.5 M sodium citrate buffer, pH 4.3) to prevent further \textit{in vitro} formation of APC-PCI complex\(^{6}\). Analysis of other types of plasma will result in higher APC-PCI values than those obtained from acid citrate plasma.

**PRINCIPLE OF THE ASSAY PROCEDURE**

The assay is a sandwich ELISA performed in microwells coated with a monoclonal antibody specific for PCI in complex with a serine protease. APC-PCI in the sample binds to the PCI-specific antibody and is detected by means of a biotinylated monoclonal antibody against PC.

The assay is a four-step procedure:

**Step 1.** Reconstituted and diluted freeze-dried calibrator, diluted plasma samples (samples should be diluted directly in the well) and any controls are incubated in precoated microwells. APC-PCI complex present in the solutions will bind to the antibody-coated wells. Unbound material is removed by washing.

**Step 2.** Biotinylated monoclonal PC detection antibody is added to each test microwell and incubated. Unbound detection antibody is removed by washing.

**Step 3.** HRP-conjugated streptavidin is added to each test microwell and allowed to form a complex with the bound biotinylated antibody. Unbound conjugate is removed by washing.

**Step 4.** A chromogenic peroxidase substrate containing tetramethylbenzidine (TMB) is added to each test microwell. The bound HRP-Streptavidin reacts with the substrate to generate a colored product. The enzymatic reaction is stopped chemically, and the color intensity is read at 450 nm in an ELISA reader. The color intensity (absorbance) indicates the concentration of APC-PCI complex originally added to each well. The results for the calibrators are used to construct a calibration curve from which the concentrations of APC-PCI complex in the plasma samples are read.
PRINCIPLE OF THE ASSAY PROCEDURE

APC-PCI antibody

Plates are precoated with primary APC-PCI antibody. The plates are ready to use.

APC-PCI complex

Samples and calibrators are added to each well and incubated.

Biotinylated PC Antibody

Biotinylated detection antibody is added to each well and incubated.

HRP-Streptavidin

HRP-Streptavidin is added to each well and incubated.

TMB Substrate

Substrate is added to each well. Develop for 10 minutes in the dark.

Stop Solution

Stop Solution is added to each well. Quantitative results are obtained by measuring the absorbances of the wells at 450 nm.

KIT COMPONENTS

<table>
<thead>
<tr>
<th>Item</th>
<th>Contents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>12 x 8 coated Microwells + Frame</td>
<td>96 wells</td>
</tr>
<tr>
<td>2</td>
<td>Sample Diluent</td>
<td>1 x 30 mL</td>
</tr>
<tr>
<td>3</td>
<td>APC-PCI Calibrator 2 glass vials each containing 1 ng freeze-dried APC-PCI</td>
<td>2 x 1 ng</td>
</tr>
<tr>
<td>4</td>
<td>25x Wash Solution Concentrate</td>
<td>1 x 40 mL</td>
</tr>
<tr>
<td>5</td>
<td>Biotinylated PC Antibody</td>
<td>1 x 12 mL</td>
</tr>
<tr>
<td>6</td>
<td>HRP-Streptavidin</td>
<td>1 x 12 mL</td>
</tr>
<tr>
<td>7</td>
<td>TMB Substrate</td>
<td>1 x 12 mL</td>
</tr>
<tr>
<td>8</td>
<td>Stop Solution</td>
<td>1 x 12 mL</td>
</tr>
</tbody>
</table>

Note: Liquid reagents contain the preservatives thimerosal, Broidox or Kathon. These may be harmful if ingested.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Adjustable micropipettes covering the range 1-1000 µL and corresponding disposable pipette tips
2. Polypropylene tubes to contain up to 1000 µL
3. Tube racks
4. Adjustable 8- or 12-channel micropipette (50-250 µL range) or repeating micropipette (optional)
5. Clean 1 L graduated cylinder
6. Deionized or distilled water
7. Cover for microplate
8. Clean container for diluted Wash Solution
9. Apparatus for filling wells during washing procedure (optional)
10. Lint-free paper towels or absorbent paper
11. Disposable pipetting reservoirs
12. Timer (60-minute range)
13. Calibrated ELISA plate reader capable of reading at 450 nm (preferably subtracting reference values at 650 or 620 nm)
14. Sodium hypochlorite (household bleach 1:10 dilution) for decontamination of specimens, reagents, and materials

PRECAUTIONS
For Research Use Only.
Not for use in diagnostic procedures.
1. This kit should only be used by qualified laboratory staff.
2. The APC-PCI calibrator was prepared from commercial APC intended for human injection, and PCI purified from human plasma. The calibrator and samples should be handled at Biosafety Level 2 as recommended for any potentially infectious sample derived from human blood in the current CDC/NIH manual “Biosafety in Microbiological and Biomedical Laboratories”. Solutions containing human plasma should be treated as potentially infectious and handled accordingly.
3. Use separate pipette tips for each sample, calibrator dilution and reagent to avoid cross-contamination.
4. Use separate reservoirs for each reagent. This applies especially to the TMB Substrate.
5. After use, decontaminate all specimens, reagents and materials by soaking for at least 30 minutes in sodium hypochlorite solution (household bleach diluted 1:10).
6. To avoid droplet formation during washing, aspirate the Wash Solution into a bottle containing bleach.
7. Avoid release to the environment. Dispose of containers and unused contents in a safe way and in accordance with national and local regulations.
8. The Stop Solution contains 0.5 M sulfuric acid and can cause irritation or burns to the skin and eyes. If contact occurs, rinse immediately with plenty of water and seek medical advice.
9. Do not interchange components from kits with different batch numbers. The components have been standardized as a unit for a given batch.
10. Hemolyzed or hyperlipemic samples may give erroneous results.
11. Dilute the plasma samples directly in the micro-wells as described below.
12. Do not touch or scrape the bottom of the micro-wells when pipetting or aspirating fluid.
13. Incubation times and temperatures other than those specified may give erroneous results.
14. Do not allow the wells to dry once the assay has begun.
15. The TMB Substrate is light sensitive. Keep away from bright light.
16. Do not reuse micro-wells or pour reagents back into their bottles once dispensed.

STABILITY AND STORAGE
1. Store the kit with all reagents at 2-8°C. Do not freeze.
2. Use all reagents before the expiry date on the kit labels.
3. Reconstituted freeze-dried calibrator stock remains stable for 24 h at 2-8°C.
4. Reconstituted freeze-dried calibrator stock can stand up to 5 thaw/freeze cycles (freeze at -20°C).
5. Diluted Wash Solution remains stable for 1 week at 2-8°C. If not all wells are to be used, dilute only the portion of Wash Solution Concentrate required.
6. For subsequent use, store unused wells in the foil pouch with the desiccant provided and reseal. Always allow foil pouch to equilibrate to room temperature before opening to avoid condensation in/on the coated micro-wells.

COLLECTION OF SAMPLES
Handle and dispose of all blood or plasma samples as if they were potentially infectious. See Precautions, sections 2, 3, 5, 6 and 7.

Determination of APC-PCI in a single sample requires 10-100 μL of plasma prepared from blood collected in acidic 0.5 M sodium citrate buffer, pH 4.3 (e.g. Biopool Stabilyte tubes, Trinity Biotech). Blood should be collected aseptically into a 5-mL tube containing 0.5 mL of the acid citrate buffer, by qualified staff using approved venepuncture techniques. The acid citrate buffer prevents in vitro formation of additional APC-PCI complex after the sample has been taken by inactivating the serine proteases that are present in the blood. Plasma should be prepared by standard techniques for clinical laboratory testing. Cap the samples and store
them at 2-8°C for assay within 24 h. If the assay cannot be performed within 24 hours or if the specimen is to be shipped, cap the specimen and keep it frozen at -20°C or below. Avoid repeated freezing and thawing. Do not use hemolyzed, hyperlipemic, heat-treated or contaminated specimens.

**PREPARATION OF REAGENTS AND SAMPLES**

1. Bring all plasma samples and reagents to room temperature (20-25°C). Mix the samples thoroughly by gentle inversion and if necessary clear visible particulate matter by low-speed centrifugation.

2. Determine the number of samples to be tested (in duplicate) plus any internal laboratory control specimens (in duplicate) plus any reagent blank wells. The precoated wells can be used as strips of 8 or as individual wells. Single wells are handled by breaking individual wells apart and placing each well in the frame at an appropriate position. Letters and notches on the wells allow the individual wells to be identified. Add 16 wells for the 8 calibrators (in duplicate). Remove the number of microwells required and replace the remainder in the foil pouch with desiccant at 2-8°C.

3. Wash Solution: dilute the 25x Wash Solution Concentrate by pouring the total contents of the bottle (40 mL) into a 1 L graduated cylinder and add distilled or deionized water to a final volume of 1000 mL. Mix thoroughly. After use, any remaining unused wash solution can be stored at 2-8°C.

4. Sample Diluent: ready to use, do not dilute further.

5. Reconstitute a vial of freeze-dried APC-PCI Calibrator with 1 mL of deionized or distilled water to produce a solution containing APC-PCI at a concentration of 1 ng/mL. Be careful when opening the vial that no freeze-dried material is lost. After adding the water close the vial again, mix gently by rotating in the hand (do not vortex), and leave the solution at 2-8°C for 10 min to ensure that all freeze-dried material has been dissolved.

6. Prepare 7 1-mL polypropylene tubes for further calibrator dilution by marking the tubes with the numbers 1 to 7. Add the appropriate volume of Sample Diluent to each tube as shown in the Table below, i.e. 160, 240, 280, 300, 310, 630 and 320 µL to tube nos. 1-7, respectively.

7. Calibrator dilutions: Dilute the reconstituted APC-PCI Calibrator (1 ng/mL) manually as shown in the Table by adding 160, 80, 40, 20, 10 and 10 µL of the 1 ng/mL reconstituted calibrator to tubes 1-6, respectively. Tube no. 7 serves as the zero calibrator, consisting of Sample Diluent alone. This procedure will result in eight calibrators of different APC-PCI concentrations: the reconstituted calibrator of 1 ng/mL plus tube nos. 1-7 containing 0.5, 0.25, 0.13, 0.063, 0.031, 0.016 and 0 ng/mL, respectively.

<table>
<thead>
<tr>
<th>Tube no.</th>
<th>APC-PCI conc. (ng/mL)</th>
<th>Dilution factor</th>
<th>Vol. of APC-PCI 1 ng/mL (µL)</th>
<th>Vol. of Sample Diluent (µL)</th>
<th>Final vol.* (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.5</td>
<td>2</td>
<td>160</td>
<td>160</td>
<td>320</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>4</td>
<td>80</td>
<td>240</td>
<td>320</td>
</tr>
<tr>
<td>3</td>
<td>0.125</td>
<td>8</td>
<td>40</td>
<td>280</td>
<td>320</td>
</tr>
<tr>
<td>4</td>
<td>0.063</td>
<td>16</td>
<td>20</td>
<td>300</td>
<td>320</td>
</tr>
<tr>
<td>5</td>
<td>0.031</td>
<td>32</td>
<td>10</td>
<td>310</td>
<td>320</td>
</tr>
<tr>
<td>6</td>
<td>0.016</td>
<td>64</td>
<td>10</td>
<td>630</td>
<td>640</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>320</td>
</tr>
</tbody>
</table>

*Only 200 µL are used, 100 µL being added to each of duplicate wells.
8. Biotinylated PC Antibody: ready to use, do not dilute further.
9. HRP-Streptavidin solution: ready to use, do not dilute further.
10. TMB Substrate: ready to use, do not dilute further.
11. Stop Solution: ready to use, do not dilute further.
12. Samples: Initial screening at a dilution of 1/10 is recommended. Dilutions lower than 1/8 should not be used. Dilute each sample with Sample Diluent directly in the microwells, as follows: Transfer 90 µL of Sample Diluent to each well by reverse pipetting. The 10 µL of sample to be diluted is transferred by direct pipetting into the Sample Diluent in the well and washed in by repeated drawing in and expulsion of the liquid in the well to obtain a 1/10 dilution of the sample. A fresh tip is used for each transfer. If assay at a dilution of 1/10 gives results that are off-scale, the sample must be reassayed at a higher dilution, as appropriate.

ASSAY PROCEDURE
1. Prepare the assay protocol, assigning the appropriate wells for setting up calibrator dilutions, samples and any internal laboratory controls in duplicate. If a reference wavelength of 650 or 620 nm is not available on the ELISA reader, a reagent blank well can be assigned. This is set up with 100 µL of Sample Diluent instead of diluted plasma and processed like the other wells.
2. Pipette 100 µL volumes of each calibrator dilution into its assigned well.
3. Pipette 90 µL volumes of Sample Diluent into each well designated for sample and add 10 µL sample. Any internal laboratory controls are set up in the same way.
4. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (300/minute).
5. Aspirate the contents of the wells and wash the wells three times with at least 300 µL of diluted Wash Solution. If washing is done manually, empty the wells by inversion and gentle shaking into a suitable container, followed by blotting in the inverted position on a paper towel. A dwell time of 1 minute before emptying is recommended for at least the last wash of the cycle. The vigor with which Wash Solution is filled into or emptied from the wells influences final color development. Manual pipetting, which may be very gentle and lead to high color development, is only recommended in the absence of alternatives such as filling the wells by immersion, using a multichannel manual washing dispenser, or using an automatic washing apparatus.
6. Dispense 100 µL of Biotinylated PC Antibody (ready to use) into each microwell. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (300/minute).
7. Wash as described above in Step 5.
8. Dispense 100 µL of HRP-Streptavidin (ready to use) into each well. A multichannel or repeating micropipette can be used. Cover the wells and incubate for 60 minutes at room temperature on a shaking platform (300/minute).
9. Wash as described above in Step 5.
10. Dispense 100 µL of TMB Substrate (ready to use) into each well. The use of a multichannel micropipette is recommended to reduce pipetting time. Cover the wells and incubate for 10 minutes at room temperature in the dark. Start the clock when filling the first well.
11. Add 100 µL of Stop Solution (ready to use) to each well, maintaining the same pipetting sequence and rate as in Step 10. Any color changes from dark blue to yellow. Mix by gentle shaking for 20 seconds, avoiding splashing. Read the wells within 30 minutes.
12. Read the absorbance of the wells at 450 nm in an appropriate microplate reader (reference wavelength 650 or 620 nm). If no reference wavelength is available, the value of the reagent blank well is subtracted from each of the other values before other calculations are performed.
SCHEMATIC OVERVIEW

1. Bring reagents to RT
2. Reconstitute freeze-dried APC-PCI Calibrator and further dilute
3. Add calibrators (100 µL) and dilute patient sample directly in the well
   - Incubate 1 h at RT
   - Wash x 3
4. 100 µL Biotinylated PC Antibody
   - Incubate 1 h at RT
   - Wash x 3
5. 100 µL HRP-Streptavidin
   - Incubate 1 h at RT
   - Wash x 3
6. 100 µL TMB Substrate
   - Incubate 10 min at RT in dark
7. 100 µL Stop Solution
8. Read at 450 nm

CALCULATION OF RESULTS
The basic principle is to construct a calibration curve by plotting the mean of duplicate absorbance values for each APC-PCI calibrator on the y-axis against the corresponding APC-PCI concentrations in ng/mL on the x-axis. The calibration curve must meet the validation requirements. The APC-PCI concentration of each diluted sample is then found by locating the point on the curve corresponding to the mean of duplicate absorbance values for the diluted sample and reading its corresponding concentration in ng/mL from the x-axis. The concentration of APC-PCI in the undiluted specimen is calculated by multiplying this result by the sample dilution factor.

This procedure can be performed manually using graph paper with linear x and y scales. A smooth curve can be drawn through the points or adjacent points can be joined by straight lines. The latter procedure may slightly overestimate concentration values between points when the curve is slightly convex to left, which is the typical finding. Although the curve may approximate to a straight line, it is both practically and theoretically incorrect to calculate and draw the straight line of best fit and to read the results from this.

The procedure can also be performed by an ELISA reader software program incorporating curve fitting procedures. The procedure of choice is to use linear x and y axes with 4-parameter logistic curve fitting.

Diluted samples that give a mean absorbance above that for the 1 ng/mL APC-PCI calibrator or below that for the 0.016 ng/mL APC-PCI calibrator are out of the range of the assay and their concentrations should be noted as >1 ng/mL and <0.016 ng/mL respectively. The corresponding concentrations in the undiluted plasma are calculated >(1 x dilution factor) ng/mL and <(0.016 x dilution factor) ng/mL, respectively. High-reading samples should be reassayed at higher dilution.

VALIDATION OF CALIBRATION CURVE
The mean absorbance for the 1 ng/mL APC-PCI calibrator should be >1.5. The mean absorbance for any APC-PCI calibrator should be higher than that for the previous APC-PCI calibrator, e.g. absorbance (0.25...
ng/mL APC-PCI) > absorbance (0.125 ng/mL). The curve should be slightly convex to the left when the results are plotted on linear axes.

**TRACEABILITY OF CALIBRATOR VALUE**
Assignment of the APC-PCI concentration of the APC-PCI solution used to freeze-dry 1 ng aliquots was by ELISA against an in-house standard, whose value was assigned by comparison in ELISA with APC-PCI complex quantified by Prof. J. Stenflo’s laboratory, University of Lund, Sweden.

**QUALITY CONTROL**
Laboratories intending to perform repeated assays should establish their own high-reading (>3 ng/mL) and low-reading (<0.2 ng/mL) control plasma samples, stored in small (e.g. 50 µL) aliquots at -20°C or below. An aliquot of each should be thawed and tested in each assay and a record kept of successive results. This serves as a control of test performance, test integrity and operator reliability. The results should be examined for drift (tendency for successive results to rise or fall) or significant deviation from the mean of previous results. Values not deviating by more than 20% from the mean of previous values can be taken to indicate acceptability of the assay. Aliquots of control plasma should not be refrozen for repeated assay once thawed, and if a further assay is performed, fresh control aliquots and fresh sample dilutions should be used.

**PERFORMANCE CHARACTERISTICS**

**Limit of detection:** The lowest concentration of APC-PCI giving an absorbance reading greater than 2 SD above the mean zero calibrator reading (n = 22) was 0.0017 ng/mL, corresponding to a plasma concentration of 0.017 ng/mL in sample diluted 1/10.

**Intraassay (within-run) reproducibility:** Four Stabilyte plasma samples were run in 8 replicates at a dilution of 1/10. The following results were obtained (CV = coefficient of variation):

**Interassay (between-run) reproducibility (different days):** Four individual Stabilyte plasma samples were run in replicates at a dilution of 1/10 in 4 separate assays to determine between-run reproducibility. The following results were obtained:

**Analytical recovery:** Two Stabilyte plasma samples were spiked with different amounts of APC-PCI complex and analyzed in the assay. Recovery was calculated as:
“Measured”/“Calculated” x 100%

<table>
<thead>
<tr>
<th>Interassay</th>
<th>APC-PCI conc. (mg/mL) mean (range)</th>
<th>Mean CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma samples 1/10</td>
<td>1.92 (1.88-1.96)</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>0.74 (0.71-0.79)</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>0.10 (0.09-0.13)</td>
<td>14%</td>
</tr>
<tr>
<td></td>
<td>3.61 (3.39-3.86)</td>
<td>4%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Interassay</th>
<th>APC-PCI conc. (mg/mL) mean (range)</th>
<th>Mean CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma samples 1/10</td>
<td>0.12 (0.11-0.13)</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>0.71 (0.68-0.74)</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>1.73 (1.59-1.92)</td>
<td>9%</td>
</tr>
<tr>
<td></td>
<td>3.32 (3.02-3.33)</td>
<td>8%</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Measured</th>
<th>Calculated</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 rec. 1</td>
<td>0.120</td>
<td>0.103</td>
<td>117%</td>
</tr>
<tr>
<td>P1 rec. 2</td>
<td>0.217</td>
<td>0.195</td>
<td>111%</td>
</tr>
<tr>
<td>P1 rec. 3</td>
<td>0.421</td>
<td>0.411</td>
<td>102%</td>
</tr>
<tr>
<td>P1 rec. 4</td>
<td>0.863</td>
<td>0.835</td>
<td>103%</td>
</tr>
<tr>
<td>Mean P1</td>
<td></td>
<td></td>
<td>108%</td>
</tr>
<tr>
<td>P2 rec. 1</td>
<td>0.120</td>
<td>0.105</td>
<td>114%</td>
</tr>
<tr>
<td>P2 rec. 2</td>
<td>0.226</td>
<td>0.197</td>
<td>115%</td>
</tr>
<tr>
<td>P2 rec. 3</td>
<td>0.421</td>
<td>0.413</td>
<td>102%</td>
</tr>
<tr>
<td>P2 rec. 4</td>
<td>0.895</td>
<td>0.837</td>
<td>107%</td>
</tr>
<tr>
<td>Mean P2</td>
<td></td>
<td></td>
<td>109%</td>
</tr>
</tbody>
</table>
LIABILITY
For Research Use Only.
Not for use in diagnostic procedures.
This kit is only intended for the in vitro determination of APC-PCI in human plasma.

The kit is only intended for use by qualified personnel carrying out research activities.

If the recipient of this kit passes it on in any way to a third party, these Instructions must be enclosed and said recipient shall at own risk secure in favor of BioPorto Diagnostics all limitations of liability herein.

BioPorto Diagnostics shall not be responsible for any damages or losses due to using the kit in any way other than as expressly stated in these Instructions.

The liability of BioPorto Diagnostics shall in no event exceed the commercial value of the kit.

BioPorto Diagnostics shall under no circumstances be liable for indirect, special or consequential damages, including but not limited to loss of profit.

Revision: PC2009-03RUO

REFERENCES
APC-PCI ELISA Kit

Catalogue number

Consult instructions for use

Use by

Use reconstituted material by

Manufacturer

Keep away from sunlight

Temperature limitation

Do not reuse

Caution, consult accompanying documents

Biological risk

Do not use if package is damaged

Freeze-dried material. Reconstitute before use

Concentrated Wash Solution. Dilute before use

FREEZE-DRIED 1 ML

WASH SOLUTION 25X