

****REPRESENTATIVE DATASHEET****

**Matched-Pair Antibody Set
for ELISA of human
Activated Protein C - Protein C Inhibitor complex**

Sufficient reagent for 5 x 96 well plates

Product #: APCPCI-EIA
Lot #: SAMPLE
Expiry Date: SAMPLE

Store at -10 to -20°C

For Research Use Only
Not for use in diagnostic procedures.

Description of APC- PCI Complex (APCPCI)

Activation of coagulation leads to the generation of thrombin which, in the presence of thrombomodulin, will activate Protein C to the enzyme activated Protein C (APC). Unless regulated, APC will exert its anticoagulant function through proteolytic inactivation of factor Va and factor VIIIa. In blood, the activity of APC is regulated in part through interaction with protease inhibitors to form inactive enzyme-inhibitor complexes. Based on physiological concentrations and the kinetics of inhibition, the primary inhibitor of APC in blood is Protein C Inhibitor (PCI, also known as plasminogen activator inhibitor-3), followed by α_1 antitrypsin (α_1 AT, also known as α_1 proteinase inhibitor) and α_2 macroglobulin.

The APC-PCI complex (APC-PCI) results when APC cleaves a scissile bond near the C-terminus of PCI, forming a covalent, 1:1 acyl enzyme intermediate with PCI with an apparent mass of 110 kDa. Calcium is not required for this interaction, but the rate of APC inhibition by PCI can be accelerated 50-fold by optimal concentrations of heparin. APC-PCI complex is cleared from circulation with a half-life of 19 minutes, presumably by serpin-enzyme complex receptors on the surface of hepatocytes.¹⁻⁴.

Principle of Sandwich-style ELISA

Affinity-purified antibody to human Protein C is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with bovine serum albumin. The plates are washed and plasma or other fluids are applied. The coated antibody will capture the APC and APC-inhibitor complexes in the sample. After washing the plate to remove unbound material, a peroxidase conjugated second antibody to PCI is added to the plate to bind to the captured APC-PCI complexes. After washing the plate to remove unbound conjugated antibody, the peroxidase activity is expressed by incubation with o-phenylenediamine (OPD). After a fixed development time the reaction is quenched with the addition of H₂SO₄ and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of APC-PCI complex present in the sample.

Supplied Materials:

1. Capture Antibody (APCPCI-EIA-C): One yellow-capped vial containing 0.5 ml of polyclonal affinity purified anti-Protein C antibody for coating plates.

2. Detecting Antibody (APCPCI-EIA-D): One red-capped tube containing 0.5 ml of peroxidase conjugated polyclonal anti-PCI antibody for detection of captured APC-PCI complex.

Note: Antibodies are supplied in a 50% (v/v) glycerol solution for storage at -10 to -20°C. Keep vials tightly capped. Do not store in frost-free freezers.

Materials Required but not Provided:

1. Coating Buffer: 50 mM Carbonate
1.59g of Na₂CO₃ and 2.93g of NaHCO₃ up to 1 litre. Adjust pH to 9.6. Store at 2-8°C up to 1 month.

2. PBS: (base for wash buffer and blocking buffer)
8.0g NaCl, 1.15g Na₂HPO₄, 0.2g KH₂PO₄ and 0.2g KCl, up to 1 litre. Adjust pH to 7.4, if necessary. Store up to 1 month at 2-8°C, discard if there is evidence of microbial growth.

3. Wash Buffer: PBS-Tween (0.1%,v/v)
To 1 litre of PBS add 1.0 ml of Tween-20.
Check that the pH is 7.4. Store at 2-8°C up to 1 week.

4. Blocking Buffer: PBS-BSA (1%, w/v)
Dissolve 2.5 g of Bovine Serum Albumin (Sigma-RIA grade) in 200 ml of PBS. Adjust pH to 7.4, if required, then make up to 250 ml with PBS. Aliquot and store frozen at -20°C.

5. Sample and Detecting Antibody Diluent: HBS-BSA-T20
5.95g HEPES (free acid), 1.46 g NaCl, 2.5 g Bovine Serum Albumin (Sigma, RIA grade) dissolved in 200 ml H₂O. Add 0.25 ml of Tween-20, check and adjust pH to 7.2 with NaOH, then make up to a final volume of 250 ml with H₂O. Aliquot and store frozen at -20°C.

6. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
2.6g Citric acid and 6.9g Na₂HPO₄ up to a final volume of 500 ml with purified H₂O. Store at 2-8°C up to 1 month.

7. OPD Substrate: (o-Phenylenediamine.2HCl) Toxic!
(5mg tablets: Sigma # P-6912). Make up immediately before use. Dissolve 5mg OPD in 12 ml substrate buffer then add 12 µl 30% H₂O₂. Do not store.

8. Stopping Solution: 2.5 M H₂SO₄
Caution: VERY CORROSIVE! GENERATES HEAT ON DILUTION! Where stock sulphuric acid is 18 Molar, add 13.9 ml to 86 ml H₂O. Store at room temperature.

9. Materials for making reference standards:
- Protein C deficient plasma in 1 mL vials (Affinity, cat# PC-LDP).
- Human Activated Protein C, Cat# APC is available from Enzyme Research Labs, South Bend, IN (tel: 574-288-2268).
- Human Protein C Inhibitor (Affinity, cat# HPCI).
- PPACK (Phe-Pro-Arg-CMK) is available from Calbiochem (www.emdbiosciences.com), Cat # 520222.

10. Other:
Microplates, 96-well Immulon 4-HBX (<http://www.labsystems.fi>)
Microplate washer (optional)
Microplate reader.

