**REPRESENTATIVE DATASHEET**

Matched-Pair Antibody Set for ELISA of human Protein C antigen (PC)

Sufficient reagent for 5 x 96 well plates

**Product #:** PC-EIA

**Lot #:** XXXX

**Expiry Date:** XXXX

**Store at -10 to -20°C**

For Research Use Only
Not for use in diagnostic procedures.

**Description of Protein C (PC)**

Protein C (PC) is a vitamin K-dependent glycoprotein produced in the liver. The concentration of PC in plasma is ~4 µg/ml (~60 nM). A deficiency of Protein C (quantitative or qualitative) is a risk factor for vascular thrombosis. Protein C is expressed as a two-chain molecule with a molecular weight of 62 kDa. The light chain (21 kDa) of PC consists of two EGF-like domains and an amino-terminal domain containing one hydroxysapatic acid and 11 γ-carboxyglutamic acid (glu) residues. These residues allow PC to bind to membranes that contain acidic phospholipids in a calcium dependent manner. The heavy chain of PC (41 kDa) consists of the catalytic domain and an activation peptide. Activation of Protein C results from cleavage at residue Arg12 in the heavy chain by a catalytic domain and an activation peptide. Activation of Protein C (APC) is a serine protease with anticoagulant activity. APC, in complex with a phospholipid membrane, calcium and the Protein S cofactor, exhibits anticoagulant activity through the proteolytic inactivation of coagulation cofactors Va and VIIIa. The primary inhibitor of APC activity in plasma is Protein C Inhibitor (PCI, also called Plasminogen activator Inhibitor-3, PAI-3) and to a lesser extent by activity in plasma is Protein C Inhibitor (PCI, also called Plasminogen activator Inhibitor-3, PAI-3).

**Principle of Sandwich-style ELISA**

Purified monoclonal antibody to PC is coated onto the wells of a microtitre plate. Any remaining binding sites on the plastic wells are blocked with an excess of bovine serum albumin. The plates are washed and plasma or other fluids containing PC are applied. The coated antibody will capture the PC in the sample. After washing the plate to remove unbound material, a peroxidase conjugated antibody to PC is added to the plate to bind to the captured PC. 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 PC in the sample.

**Supplied Materials:**

1. **Capture Antibody (PC-EIA-C):** One yellow-capped vial containing 0.5 ml of purified monoclonal anti-PC antibody for coating plates.

2. **Detecting Antibody (PC-EIA-D):** One red-capped vial containing 0.5 ml of peroxidase conjugated polyclonal anti-PC antibody for detection of captured PC.

**Materials Required but not Provided:**

This paired antibody set has been optimized for performance using the buffers and conditions described below. Most reagents are available as part of the VisuLize™ Buffer Pak (see Related Products section) or may be prepared as described below.

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 K2HPO4 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 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) Toxel (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.


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**VisuLize™ Buffer Pak**

- **Coating Buffer 1:** 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.
- **Blocking Buffer 1:** 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.
- **Sample Diluent 1:** 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.

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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 K2HPO4 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.

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7. **OPD Substrate:** (o-Phenylenediamine.2HCl) Toxel (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.


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**Supplied Materials:**

1. **Capture Antibody (PC-EIA-C):** One yellow-capped vial containing 0.5 ml of purified monoclonal anti-PC antibody for coating plates.

2. **Detecting Antibody (PC-EIA-D):** One red-capped vial containing 0.5 ml of peroxidase conjugated polyclonal anti-PC antibody for detection of captured PC.

**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.
**Assay Procedure:**

1. **Coating of plates:**
   Dilute the capture antibody 1/100 in coating buffer (preferably in a polypropylene tube) and immediately add 100 µl to every well in the plate. Incubate overnight at 2-8°C or for 2 hours at 22°C.

2. **Blocking:**
   Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 90 minutes @ 22°C. Wash plate X 3 with wash buffer.

3. **Samples:**
   Reference plasma is diluted 1/100 (100%) then serial 1/2’s down to 1/3200 (3.13%). Sample plasmas are diluted 1/200, 1/400 & 1/800. All dilutions are made in HBS-BSA-T20 sample diluent. Apply 100 µl/well and incubate plate @ 22°C for 90 minutes. Wash plate X 3 with wash buffer.

4. **Detecting Antibody:**
   Dilute the detecting antibody 1/100 in HBS-BSA-T20 sample diluent and apply 100 µl to each well. Incubate plate @ 22°C for 90 minutes. Wash plate X 3 with wash buffer.

5. **OPD Substrate:**
   Apply 100 µl of freshly prepared OPD substrate to every well. Allow colour to develop for 10-15 minutes then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at a wavelength of 490 nm.

**Calculation of Results:**

The construction of a proper reference curve is of no less importance than any other aspect of the assay. A reference curve should be constructed by plotting the known concentration of standards versus absorbance. This can be done manually using graph paper, or by using curve-fitting computer software. In our experience, the dose response curves of most immunoassays tend to be sigmoid in shape. Although linear regions can be identified within the curve, the best overall fit is often obtained using an algorithm that provides a weighted theoretical model of fit throughout the entire curve, such as a 4-parameter or 5-parameter logistic curve fit 4,5. In general, the simplest model that defines the concentration-response relationship should be used 6. The “back-fit” test is a simple and reliable method to determine if a curve-fitting method is appropriate. In this test, the apparent concentrations for the absorbance values of each standard point are read from the reference curve. The derived values are compared to the assigned values. An appropriate curve fitting method will produce derived values that closely match assigned values throughout the range of the curve, within user-defined limits 5.6 In the quality control of this product we have determined that under the conditions described above, a reference curve that is constructed using serial dilutions of normal pooled plasma, will produce a correlation coefficient (R²) of at least 0.980 using a log-log fit, and an R² of at least 0.990 using a 4-parameter logistic curve fit algorithm. However, the performance characteristics of in-house assays developed using this product in other laboratories may vary slightly from ours. Different curve fitting methods may be employed but we recommend that the back-fit test be applied as evidence that the fitting method is appropriate.

**References:**


**Related Products:**

- Cat #: EIA-PAK-1 VisuLize™ Buffer Pak: 5 plates, buffers, substrate
- Cat #: EIA-CSA-1 VisuCal™ Antigen Calibrator, 1 x 1ml
- Cat #: EIA-CSA-5 VisuCal™ Antigen Calibrator, 5 x 1ml
- Cat #: GAPC-IG Goat anti-human Protein C, whole IgG from antiserum
- Cat #: GAPC-AP Goat anti-human Protein C, affinity purified IgG
- Cat #: SAPC-IG Sheep anti-human Protein C, whole IgG from antiserum
- Cat #: SAPC-AP Sheep anti-human Protein C, affinity purified IgG
- Cat #: SAPC-HRP Sheep anti-human Protein C, IgG-peroxidase
- Cat #: MAPC-IG Murine anti-human Protein C, IgG from ascites
- Cat #: APCAT-EIA Paired antibody ELISA of APC-a,AT complex, 5 x 96 wells
- Cat #: APCPCI-EIA Paired antibody ELISA of APC-PCI complex, 5 x 96 wells
- Cat #: PC-DEP Human plasma deficient in PC, immune depleted
- Cat #: PC-LDP Human plasma deficient in PC, lyophilized

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