Version 24-04/25

User's Manual

Human Protein C Matched Antibody Pair

REF **ABPR-L019**

Σ **5 x 96 tests**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Human Protein C (PRROC)

General Description

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 hydroxyaspartic acid and 11 γ-carboxyglutamic acid (gla) 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 complex of thrombin and a cell surface cofactor thrombomodulin. The activation of PC is associated with the release of a small activation peptide (2-3 kDa, called Protein C peptide, or PCP) from the N-terminal of the heavy chain. Activated 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 α1 Antitrypsin and α2 macroglobulin. The inhibitory activity of PCI is stimulated approximately 10 fold by heparin.

Principles of Testing

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 second 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.

Reagents And Materials Provided

1. Capture Antibody (yellow): 0.5 ml of purified monoclonal anti-PC antibody for coating plates.
2. Detecting Antibody (red): 0.5 ml of peroxidase conjugated polyclonal anti-PC antibody for detection of captured PC.

Note: Reagents are sufficient for at least 5×96 well plates using recommended protocols. 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 Supplied

1. Coating Buffer: 50 mM Carbonate
2. PBS:(base for wash buffer and blocking buffer)
3. Wash Buffer: PBS-Tween(0.1%, v/v)
4. Blocking Buffer: PBS-BSA(1%, w/v)
5. Sample Diluent: HBS-BSA-T20
6. Substrate Buffer: Citrate-Phosphate buffer pH 5.0
7. OPD Substrate:(o-Phenylenediamine. 2HCl) TOXIC!
8. Stopping Solution: 2.5 M H2SO4
9. Other: Microplates; Microplate washer; Microplate reader.

Storage

-10 to -20°C

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 at 22°C. Wash plate 3 times 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 and 1/800. All dilutions are made in HBS-BSAT20 sample diluent. Apply 100 µl/well and incubate plate at 22°C for 90 minutes. Wash plate 3 times 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 at 22°C for 90 minutes. Wash plate 3 times 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.