Version 09-05/25

**User's Manual**

# Human Protein C Inhibitor (PCI) Matched Antibody Pair

REF **ABPR-L024**

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**

**Address: 45-1 Ramsey Road, Shirley, NY 11967, USA**

**Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe)** **Fax: 1-631-938-8221**

**Email: info@creative-diagnostics.com** **Web: www.creative-diagnostics.com**

# PRODUCT INFORMATION

## Intended Use

This antibody pair set comes with matched antibody pair to detect and quantify protein level of Human SERPINA5

## General Description

Protein C Inhibitor (PCI), also known as Plasminogen Activator Inhibitor 3 (PAI3), is a member of the SERPIN family of proteinase inhibitors. It is produced in the liver as a single chain glycoprotein (mass of 57 kDa) and circulates in plasma at a concentration of 5 µg/ml (~90 nM). PCI is also found in urine in lower concentrations of 250 ng/mL (~0.4 nM). PCI is the primary inhibitor of activated Protein C (APC) in plasma but demonstrates a relatively broad specificity, also inhibiting thrombin, FXa, F.XIa, kallikrein, tPA, urokinase, prostate specific antigen, acrosin, chymotrypsin and trypsin. The preferred enzyme target for PCI appears to be thrombin and this interaction is increased by more than 100 fold in the presence of thrombomodulin. Like ATIII and HCII, the inhibitory activity of PCI towards some of these enzymes is stimulated by high concentrations of heparin (5 U/ml) which can accelerate the rate of inactivation as much as 50 fold. Enzyme inhibition by PCI occurs through proteolytic cleavage at Arg354-Ser355 and subsequent rapid formation of a stable, inactive 1:1 enzyme-PCI complex. Interaction with APC results in an SDS-stable APC-PCI complex of 102 kDa.

## Principles of Testing

Affinity-purified antibody to PCI 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 containing PCI are applied. The coated antibody will capture the PCI 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 PCI. 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 $H_2SO_4$ and the colour produced is quantified using a microplate reader. The colour generated is proportional to the concentration of PCI present in the sample.

## Reagents And Materials Provided

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

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.
2. **Blocking:** Empty contents of plate and add 150 µl of blocking buffer to every well and incubate for 60 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 and 1/400. All dilutions are made in HBS-BSA-T20 sample diluent. Apply 100 µl to each well and incubate for 90 minutes at 22°C. 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 5-10 minutes then stop colour reaction with the addition of 50 µl/well of 2.5 M H2SO4. The plate can be read at wavelength of 490 nm.