# TECHNOZYM® PCI Actibind ELISA Kit

For research use only REF TC16100

3050091-010

Protein C Inhibitor (PCI) is a member of the serine protease inhibitor (Serpin) superfamily with homology to alpha-1-antichymotrypsin, alpha-1-antitrypsin, antithrombin III, ovalbumin and angiotensinogen. PCI with an apparent molecular weight of 57 KD has been described in a variety of biological fluids including blood plasma (4µg/mL), urine (250ng/mL) and seminal plasma (200µg/mL). Glycosaminoglycan (GAG) dependent PCI inhibits activated protein C (APC), two-chain urokinase (u-PA), two-chain tissue plasminogen activator (t-PA), thrombin, factor Xa, and factor XIa in reactions stimulated by heparin. However it has recently been shown that the PCI-tissue kallikrein interaction is inhibited by heparin. Glycosaminogylcans may therefore regulate the enzyme specificity of PCI.
PCI inhibits its target proteases by forming SDS stable 1:1 complexes. Upon complex formation the reactive site peptide bond of the inhibitor is cleaved by the protease and the carboxy-terminal peptide is released from the inhibitor. Depending on the target protease, complexes dissociate more or less slowly and cleaved inactive PCI (Mr=54,000) and active enzyme are released.
Although PCI has been shown to inhibit several enzymes, its precise physiological role has yet to be defined. However, the fact that PCI has been determined in high concentrations in a variety of biological fluids indicates its importance as a physiological serine protease inhibitor whether it be as an inhibitor of a specific serine protease in vivo or as a general inhibitor functioning to protect tissues from protease action.
Low values of both Protein C antigen and functional activity of PCI have been determined in patients with disseminated intravascular coagulation and low antigen values in liver disease and high PCI activity in survivors of MI. uPA-PCI complexes are formed in patients undergoing thrombolytic therapy with u-PA when the concentration of u-PA exceeds the inhibitory capacity of plasminogen activator inhibitor-1 (PAI-1), the primary inhibitor of u-PA in vivo.

## APPLICATION

The Actibind PCI can be used as a quantitative assay for the determination of active antigen levels of PCI in patients with disseminated intravascular coagulation and in atheriosclerotic disease.

## TEST PRINCIPLE

The TC Actibind PCI test is a solid phase enzyme immunoassay in which an anti-u-PA monoclonal antibody that does not interfere with the active site on the urokinase antigen molecule is coated onto a plastic microtitre plate. Urokinase is incubated on the plate leaving its active site accesible for complex formation with active PCI contained in the sample. Following the binding of the sample the plate is washed and enzyme-labelled (POX = horseradish peroxidase) monoclonal anti-PCI which recognises another site on the active PCI antigen is incubated on the plate. The quantity of POX which binds is proportional to the quantity of active PCI antigen i.e. non-complexed antigen contained in test samples. Unbound POX-Ab is washed away and a substrate which reacts with the peroxidase enzyme is added, leading to a colour change proportional to the amount of enzyme bound. The enzyme reaction is stopped after a specific incubation time. The absorbances of the wells are then measured and the values obtained are used to construct a standard curve from which sample values can be extrapolated.

## KIT COMPONENTS

Determinations: 42 samples in duplicate

1. **MICROTITRE PLATE**
   12 x 8 well plastic microtitre strips precoated with monoclonal anti urokinase coating antibody.
2. **STANDARD**
   1 x lyophilized pooled human plasma
3. **UROKINASE**
   1 x lyophilized urokinase 800U/vial
4. **POX-ANTIBODY**
   1 x conjugated monoclonal anti PCI antibody (concentrated), blue colour
5. **SAMPLE DILUTION BUFFER - (white cap)**
   2 x 20 mL 2.5-fold concentrated PBS with BSA
6. **POX DILUTION BUFFER - (white cap)**
   1 x 12 mL PBS with BSA.
7. **SUBSTRATE – (green cap)**
   1 x 12 mL TMB (Tetramethylbenzidine) in substrate buffer containing $H_2O_2$. Ready to use
8. **STOP SOLUTION - (red cap)**
   1 x 15 mL 0.5 M Sulphuric Acid Ready to use
9. **WASH BUFFER - (blue cap)**
   1 x 20 mL 12.5-fold concentrated PBS with Tween 20
10. **PCI ACTIBIND HIGH CONTROL**
    1 x lyophilized pooled human plasma for lot-specific concentration see label on vial

## ADDITIONAL MATERIAL

1. Micropipettes and a multichannel micropipette or multistepper pipette, pipette tips
2. Glass or plastic test tubes for diluting the samples.
3. Laboratory bottles or beakers and graduated cylinders for diluting wash and dilution buffer
4. Distilled or deionised water
5. Absorbent paper towels
6. Microtitre plate washer (alternatively, washing can be performed manually using a multichannel pipette)
7. Microtitre reader equipped with a 450 nm filter and, if possible, a 620 nm reference filter
8. Incubator (37 °C)

## SAMPLES

Use fresh EDTA or citrated plasma samples.. Centrifuge the blood within 30minutes after the puncture at 2000g for 30min. at 4°C (preferably in a cold centrifuge with swing out rotor). Immediately after centrifugation, plasma should be carefully pipetted off. Plasma aliquots should be stored at a temperature below -30°C. The total time between blood collection and plasma freezing should not exceed 90min. Thawing and refreezing of plasma aliquots is not recommended.
Thawing for assay is achieved rapidly using a waterbath at 37°C. After thawing, plasma samples are placed in a crushed ice-water mixture until analysis.

## TEST PERFORMANCE

## PREPARATIONS AND STABILITY OF REAGENTS

All reagents must be at room temperature before use.

| Component | Volume/ bottle | Additions | Bench Stability |
|---|---|---|---|
| Dilution buffer | 20 mL | 30 mL distilled $H_2O$ | 4-8 weeks at 2..8 °C |
| Wash buffer | 20 mL | 230 mL distilled $H_2O$ | 4-8 weeks at 2...8 °C |
| POX AB (concentrate) | 0,3 mL | Dilute 1+50 with POX dilution buffer: **for 8 test wells: mix 20 µl conjugate with 1000 µl POX dilution buffer** | working dilution: RT: 4 hours |
| Standard and Controls | - | 1 mL distilled $H_2O$ | Aliquot –70 °C: 8 weeks RT: 4 hours |
| Urokinase | - | 12 mL dilution buffer | Aliquot –70 °C: 8 weeks RT: 4 hours |

RT = Room Temperature
POX AB = Peroxidase conjugated Antibody

## MICROTITRE PLATE

Reseal unused strips in aluminium foil bag. Store at 4 °C for up to 8 weeks.

## SAMPLE DILUTIONS

Dilute control 1: 5
Dilute samples 1: 2

**60 µL control + 240 µL dilution buffer**
**150 µL sample + 150 µL dilution buffer**

## STANDARD DILUTIONS

Prepare serial dilutions (1:2 to 1:8) of the standard in dilution buffer according to the following protocol. C = concentration according to label

| Tube | Dilution factor | | Dilutionbuffer | Conc. % of normal plasma |
|---|---|---|---|---|
| A | NT | 0.25 mL Standard | | C |
| B | 1:2 | 0.25 mL Standard | 0.25 mL | C/2 |
| C | 1:4 | 0.25 mL from B | 0.25 mL | C/4 |
| D | 1:8 | 0.25 mL from C | 0.25 mL | C/8 |
| E | - | - | 0,25 ml | 0 |

## ASSAY PROCEDURE

Over view of assay procedure

| **Time table<br>Summary of procedure** | | **time required** | **Temp.** |
|---|---|---|---|
| Preparation of plate:<br>**binding of urokinase** | 100 µL | 1 hour | 37°C |
| wash 5 times | 200 µL | | |
| Reagent, Standard<br>Sample handling | | 1-2 hours | |
| **1. Sample - incubation** | 100 µL | 2 hours | 37°C |
| wash 5 times | 200 µL | | |
| **2. POX AB – incubation** | 100 µL | 2 hours | 37°C |
| wash 5 times | 200 µL | | |
| **3. Substrate - incubation** | 100 µL | 15 minutes | RT |
| **Stop solution** | 100 µL | | |
| **Read absorbances** | 450 nm | | |
| **Time total: min.** | | **6½-7½ +** | |

1. **MICROTITRE PLATE PREPARATION**
   Pipette 100µL of the reconstituted urokinase to each well. Incubate the plate for 1 hour at 37 °C.
2. **WASH PLATE**
   Wash required strips by adding 200 µL of wash buffer to the wells and tip out the contents. Wash the strips four times further with wash buffer. Tap strips on absorbant paper and make sure the wells are completely dry.
3. **SAMPLE/ STANDARD/CONTROLS ADDITION**
   Pipette 100 µL of the samples/standard/controls into separate wells. For zero standard, pipette buffer only into the well. Running standard /sample/controls in duplicate is recommended.
4. **SAMPLE INCUBATION**
   Cover the plate with a fresh plastic foil and incubate for 2 hours at 37 °C.
5. **WASH PLATE**
   Wash five times as described in step 2.
6. **POX ANTIBODY ADDITION**
   Pipette 100 µL of POX antibody to all wells containing sample, controls or standards.
7. **POX ANTIBODY INCUBATION**
   Cover the plate with a fresh plastic foil and incubate for 2 hours at 37 °C.
8. **WASH PLATE**
   Wash five times as described in step 2.
9. **SUBSTRATE**
   Pipette 100 µL of TMB substrate to all wells. Incubate for 15 minutes at room temperature. There should be a colour change from clear to blue.
10. **STOP**
    Pipette 100µL of stop solution to all wells. The colour of the endproduct should be yellow.
11. **READ**
    Measure absorbances at 450 nm (with 620 nm reference filter if available). Read absorbances within one hour after the addition of the stop solution.

## NOTES

Be sure to prepare all reagents before proceeding with the assay. It is critical to keep the time necessary for pipetting standards and samples to a minimum and avoid delays.
Be sure to wash the plate thoroughly and completely remove any residual wash buffer after each wash cycle. Insufficient washing can lead to erroneously high values and incomplete removal of wash buffer to irregularities due to the dilution of added reagents
As mentioned use a multistepper to add peroxidase conjugate, TMB substrate and stop solution.

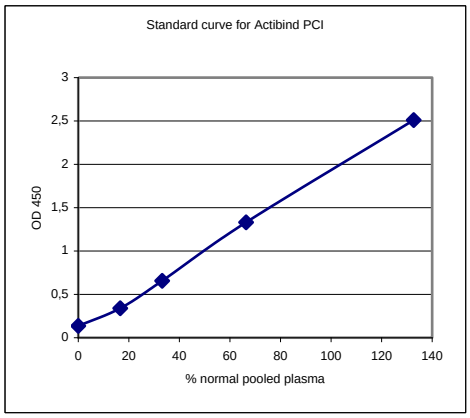
## TEST EVALUATION

### CONSTRUCT A STANDARD CURVE

Construct a graph of the standard curve. An example of a typical curve is given below. A standard curve must be constructed with each assay.

### DETERMINATION OF SAMPLE CONCENTRATION

Locate the absorbance for each sample on the curve and read the corresponding value from horizontal axis. Do not forget to multiply by the dilution factor (5) for the control and (2) for the samples, prediluted before adding to the plate.




## EVALUATION OF RESULTS

The results are measured as a percentage of normal pooled human plasma. The validity of the test may be checked on the basis of the calculated control values.
Lowered PCI values have been detected in patients with disseminated intravascular coagulation and liver disease, whereas increased levels have been detected in survivors of MI.

## TEST CHARACTERISTICS

This test system measures active PCI antigen.
The inter- and intra-assay variations are less than 10 % and 5 %, respectively

## STABILITY AND STORAGE

All the components of the kit should be stored at 2…8°C and can be used until the indicated expiry date on the vial labels. For storage of samples see above.

## SPECIAL PRECAUTIONS

Potentially biohazardous material. Donor plasma used in this kit was tested by internationally approved methods for the presence of antibodies to HIV and hepatitis B virus and found to be negative. However, all human blood products should be handled as potentially infectious material.
The stop solution ($H_2SO_4$) can cause skin irritations, wash with plenty of water if spilt on the skin.

## LITERATURE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