

**ACTICLOT® Protein C** 3 x 15 test kit for the determination of Plasma Protein C Activity Via a Clotting End-Point

Product No. ACC-45

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For *in vitro* diagnostic use.

### INTENDED USE

ACTICLOT® C is intended for the measurement of Protein C activity in human plasma via an end-point clotting assay.

### PRINCIPLE

Protein C is a vitamin K-dependent anticoagulant protein that normally circulates as an inactive zymogen. After activation, Protein C inactivates factors V and VIII thus prolonging the clotting time. While Protein C can be activated by thrombin, the rate of activation *in vitro* is slow. Under such conditions Protein C inhibitor protein inactivates Protein C as fast as it is activated.

PROTAC™ is a rapid Protein C activator derived from the venom of the viper *Agkistrodon contortrix* (Reference 1). Under the conditions described below, PROTAC converts human Protein C to the active protease within 5 minutes (Reference 2). In this assay PROTAC is co-lyophilized with an APTT reagent to form a reagent that activates both Protein C and the contact factors of the intrinsic pathway. With this reagent, the clotting time of normal plasma is very long (>100 seconds) while that of Protein C deficient plasma is essentially the same as the APTT (approx. 30-40 seconds). When patient plasma is mixed with Protein C deficient plasma the prolongation of the clotting time is proportional to the amount of Protein C in the patient plasma.

The following assay works well with semi-automated, optical or mechanical coagulation timers or with automated instruments.

### REAGENTS

- Acticlot Activator:** 3 vials each containing 1.5 units PROTAC co-lyophilized with APTT reagent (rabbit brain cephalin and colloidal silica activator).
- Protein C Deficient Plasma:** 3 vials each containing 1.5 mL of freeze-dried human plasma substrate that has been artificially depleted of Protein C by adsorption on an immobilized immunospecific goat polyclonal antibody to human Protein C.
- Protein C Control Plasma:** 3 vials each containing 0.5 mL of freeze-dried normal human plasma that has been assayed for Protein C antigen and activity against the 1st International Standard for Protein C, 86/622.
- Dilution Buffer:** 3 vials each containing 5 mL of a 10-fold concentrate. After dilution, the buffer contains 0.12 M NaCl, 0.03M imidazole pH 7.35. The buffer also contains protamine sulfate to neutralize up to 1 USP unit/ml heparin in the plasma sample.

### REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- 0.025M calcium chloride solution
- clot timer
- 50 mL graduated cylinder
- variable volume pipettor (100-1000 µL)
- one cycle log-log graph paper

### REAGENT RECONSTITUTION AND HANDLING

Unreconstituted reagents are stable until the expiration date indicated on the label when stored at +2 - +8°C.

- Acticlot Activator:** Reconstitute with 1.5 mL purified water.  
Stability at -20°C: 3 months  
at 2-8°C: 48 hours  
at 37°C: 4 hours  
Some automated equipment may require a larger Activator volume in order to adequately fill the reagent reservoir and pump tubing. In this case, reconstitute all three vials of Activator provided and pool to obtain a reagent volume of 4.5 mL after use. The remaining contents of the reservoir and pump tubing may be returned to the vial, capped, frozen at 20°C and reused. Activator can be frozen and thawed virtually without loss of activity.
- Protein C Deficient Plasma:** Reconstitute with 1.5 mL purified water. Let stand at room temperature for 20 minutes for complete dissolution. Use immediately or store on melting ice until use.
- Protein C Control Plasma:** Reconstitute with 0.5 mL purified water. Let stand at room temperature for 20 minute for complete dissolution, Use immediately or store on melting ice until use. Use as a quality control reagent when performing the assay.
- Dilution Buffer:** Dilute to 50 mL with purified water.  
Stability at room temperature: 1 week  
at +2 - +8°C: 1 month

### WARNING

The source material for this reagent has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA Approved methods. As no known test method can provide complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, this reagent should be handled as recommended for any potentially infectious human specimen.

The Acticlot Dilution Buffer contains sodium azide that may react with lead or copper plumbing to form highly explosive metal azides. Materials discarded into a sink should be flushed with a large volume of water to prevent azide build-up.

### SPECIMEN COLLECTION

Nine volumes of blood are collected in 1 volume of 0.1M trisodium citrate and centrifuged at 3000 x g for 10 minutes. Plasma should be stored at 2-8°C and assayed within 24 hours. Alternatively, plasma may be stored at -20°C for 1 month and thawed once at 37°C, 30 minutes before use.

### ASSAY CALIBRATION

Pooled normal plasma from at least 10 normal donors that has been collected in the same manner as plasma to be tested should be used for preparation of Protein C calibration standards. Alternatively, dilutions of the Protein C Control Plasma may be used to prepare the assay calibration standards. In this event, however, a different lot of Protein C Control Plasma (Cat. No. 247) should be used as quality control plasma.

Prepare plasma Protein C calibration standards and patient plasma samples as follows:

100% Standard	100 µL pooled plasma	+	400 µL Dilution Buffer
50% Standard	250 µL 100% control	+	250 µL Dilution Buffer
25% Standard	250 µL 50% control	+	250 µL Dilution Buffer
12.5% Standard	250 µL 25% control	+	250 µL Dilution Buffer
*Patient samples	50 µL patient plasma	+	450 µL Dilution Buffer

Store dilutions on melting ice and use immediately after preparing.

\* Patients with lupus-type anticoagulants should be tested at multiple dilutions as artifactually high Protein C levels could be inferred from prolonged clotting times. Similarly, multiple patient plasma dilutions should be used when the patient plasma has an abnormally high factor VIIIc level. In this case artifactually low Protein C levels due to shortening of the clotting time of the Protein C Deficient Plasma may be obtained.

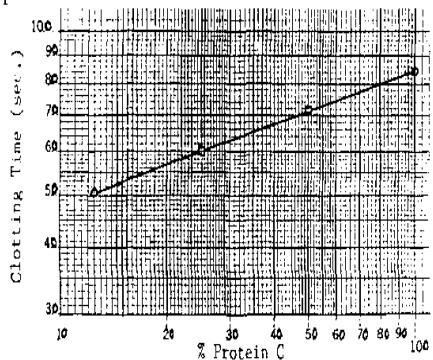
#### ASSAY PROCEDURE

1. Reconstitute reagents as described above.
2. Transfer Activator and calcium chloride to 37°C reagent wells in clot timer if the instrument is manual. In the case of automated instruments prime reagent delivery tubing, set activation time to 5 minutes and maximum end-point time to 100 seconds.
3. Prepare dilutions as described above.
4. To a coagulation cuvette:
  - Add 0.1 mL Protein C Deficient Plasma + 0.1 mL standard dilution
  - Incubate for 2 minutes at 37°C
  - Add 0.1 mL Acticlot Activator
  - Incubate for 5 minutes at 37°C
  - Add 0.1 mL Calcium Chloride (0.025 M)
  - Start clot timer and note clotting time
  - Obtain duplicate determinations for each plasma dilution.
5. Repeat step 4 for patients' plasma dilutions in duplicate.
6. Using one-cycle log-log graph paper, plot % Protein C activity of the calibration standards on the x-axis vs. mean clotting time on the y-axis. Draw the line of best fit between the resulting points. For unknown samples determine the % Protein C by interpolating from the standard curve and multiplying the result by two to correct for dilution. In the case of patients with lupus anticoagulants or abnormally high Protein C activity where multiple patient dilutions were used, correct Protein C level for the dilution. Corrected Protein C levels from at least two dilutions must agree.

#### SAMPLE CALIBRATION CURVE

The calibration curve shown in Figure 1 is an example for only. A calibration curve must be generated each time the assay is performed, preferably during the same run as patient samples are tested.

Figure 1



#### PERFORMANCE CHARACTERISTICS

In a clinical study comparing ACTICLOT C to a Protein C ELISA (Reference 3), the following results were obtained:

#### PLASMA PROTEIN C CONCENTRATION

(% of pooled normal plasma, mean + s.d.)

Population	n	ACTICLOT C	ELISA
Normal	40	89.0 ± 17.0	94.0 ± 16.0
DIC	10	29.4 ± 11.9	34.2 ± 13.0
Liver Disease	10	18.6 ± 9.6	20.1 ± 14.6
Congenital Def.	10	37.9 ± 7.1	45.0 ± 8.1
Heparin	10	93.7 ± 16.1	93.5 ± 14.2
Coumadin	20	23.2 ± 9.0	57.7 ± 15.7
*Sick Neonates	12	21.9 ± 5.3	19.3 ± 8.0

\* e.g. respiratory distress syndrome, sepsis, thrombosis, renal failure

Correlation between ACTICLOT C and ELISA (coumadin patients on coumadin not included):

Regression Line	Correlation Coefficient	Standard Error of Estimate
$y = 0.93x + 0.0014$	0.952	0.086

The coefficient of variation of the assay has been determined using plasma samples prepared by mixing plasma that has been totally immunodepleted of Protein C with normal pooled plasma to obtain Protein C levels of 10%, 50% and 100%.

#### Protein C Level Intra-Assay C.V. Inter-Assay C.V.

100%	5.9%	2.4%
50%	4.7%	3.9%
10%	9.1%	9.3%

#### QUALITY CONTROL

Use Protein C Control Plasma provided in the kit for quality control of the assay. If the Protein C Control Plasma has been used to construct the calibration curve for the assay, then a different lot of Protein C Control Plasma (Cat. No. 247) should be used for quality control.

#### REFERENCES

1. Stocker K, Fischer H, Mejer J, Brogill M and Svendsen L: Characterization of the protein C activator Protac from the venom of the Southern Copperhead (Agkistrodon contortrix) snake. *Toxicol.* 1987, **25**: 239-252.
2. Martinoli JL and Stocker K., Fast functional protein C assay using Protac, a novel protein C activator. *Thromb. Res.* 1986, **43**: 253- 264.
3. United States FDA 510(k) Notification, Document No. K884941, 1987.