

# CoaChrom® Prothrombin Chromogenic Assay for Prothrombin

Ref.no. 400001

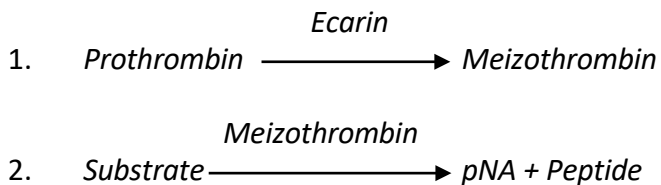


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Not For Use in Diagnostic Procedures  
For *in vitro* Use Only

CoaChrom® Prothrombin is an *in vitro* assay for the quantitative determination of Prothrombin in citrated plasma or any other biological fluid with a chromogenic assay, using a manual or automated assay protocol.

## ASSAY PRINCIPLE

Prothrombin is activated to Meizothrombin by the snake venom enzyme Ecarin from *Echis Carinatus*. After a fixed incubation time, the amount of Meizothrombin formed is measured with a Thrombin selective chromogenic Substrate, which is cleaved by Meizothrombin. The absorbance recorded at 405nm is proportional to the Prothrombin activity in the sample.



## REAGENTS

The reagents should be stored at 2-8°C until reconstituted.

**1. Tris-BSA Buffer, 50 mL** 2 vials  
Ready-to-use buffer for sample dilution, containing 0.05 mol/L Tris-HCl pH 7.5, I=2.0 with NaCl and BSA. When open and protected from any contamination, the buffer is stable for up to 8 weeks at 2-8°C.

**2. Prothrombin-Activator-Diluent, 20 mL** 1 vial  
Ready-to-use buffer for dilution of Ecarin, containing 0.05 mol/L Tris HCl pH 7.9, I=0.15 with NaCl, BSA, polyethylene glycol and a fibrin polymerization inhibitor. When open and protected from any contamination, the diluent is stable for up to 8 weeks at 2-8°C.

**3. Ecarin, 5 mL** 1 vial  
Reconstitute with 5.0 mL sterile, deionized water. Stable for up to 7 days at 2-8°C or freeze in aliquots for up to 3 months at -20°C.

## 3a. Ecarin Working Solution

Dilute 1 part Ecarin (Reagent 3) with 2 parts Prothrombin-Activator-Diluent (Reagent 2) to obtain a Ecarin working solution. The solution is stable for up to 8 hours at 20-25°C or 7 days at 2-8°C.

**4. Chromogenic Thrombin Substrate** 1 vial  
25 mg of chromogenic Thrombin Substrate H-D-Phe-Pip-Arg-pNA·2HCl. Reconstitute with 13 mL of sterile, deionized water to obtain a 3 mmol/L solution. Stable for up to 6 months at 2-8°C.

## SPECIMEN COLLECTION

Blood (9 volumes) is mixed with 0.1 mol/L sodium citrate (1 volume) and centrifuged at 2000 x g for 20 minutes at 20-25°C. Separate plasma carefully from blood cells. Perform the analysis within 24 hours when plasma is stored at 2-25°C. Alternatively, freeze aliquots ≤ 1mL at -20°C or below. Perform the analysis of frozen samples within two months when stored at -20°C or within one year when stored at -70°C or below. No significant loss of Prothrombin activity occurs upon freezing once, provided freezing is made in small aliquots (< 1 mL) and thawing is performed in a water bath at 37°C.

## SAMPLE AND STANDARD DILUTIONS

### Standards

Calibrated normal plasma is diluted 1:23-1:160 to provide standard concentrations of 25-175%. The following table provides a **suggestion** of standard dilutions.

Standard Dilution	Prothrombin Activity
1:23	175%
1:29	138%
1:40	100%
1:80	50%
1:160	25%

## **Samples**

Plasma Samples are diluted 1:40 in Tris-BSA Buffer (Reagent 1) for application on microplate and 1:80 for application on automatic analyzers.

## **MICROPLATE ASSAY PROCEDURE**

<b>Standard/Sample dilution</b>	<b>50 µL</b>
<b>Incubate at 37°C</b>	<b>2-4 min</b>
<b>Ecarin working solution (37°C)</b>	<b>50 µL</b>
<b>Incubate at 37°C</b>	<b>3 min</b>
<b>Chromogenic Substrate (37°C)</b>	<b>50 µL</b>
<b>Read kinetically or incubate at 37°C</b>	<b>2 min</b>
<b>Acetic acid, 20%</b>	<b>50 µL</b>

Determine the absorbance difference A405nm - 490nm for the standard dilution and the samples. Draw a standard curve from the absorbance obtained for the standard dilutions. Read the prothrombin activity for the samples from the standard curve.

## **INTERFERENCE AND LIMITATIONS**

No influence in the assay is obtained from variation of Antithrombin activity in the range 50-150% of normal. Since Meizothrombin is formed and measured, no influence in the assay is obtained from heparin levels  $\leq 1$  IU/mL plasma. Since Ecarin also activates Decarboxyprothrombin, which is produced during oral anticoagulant therapy with anti-vitamin K drugs, plasma from patients undergoing such treatment should not be analyzed with this method.

## **REFERENCES**

1. Lane DA, Mannucci PM, Bauer KA, Bertina RM, Bochkov NP, Boulyjenkov V, Chandy M, Dahlbäck B, Ginter EK, Miletich Jp, Roosendaal FR, Selingsohn U. Inherited Thrombophilia: part 1. Thromb Haemost 76, 651-662 (1996).



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