CoaChrom® Prothrombin

Chromogenic Assay for Prothrombin

Ref.no. 400001

For Research Use Only
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.

1. *Prothrombin* —(*Ecarin*)→ *Meizothrombin*

2. *Substrate* —(*Meizothrombin*)→ *pNA + Peptide*

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

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

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 ≤ 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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