

HEMOCLOT – THROMBIN (HUMAN)

ACK001K

Kit for the Thrombin Time on plasma,
using highly purified human Thrombin
6 x 20 tests



Manufactured By: HYPHEN BioMed

For in vitro use only



For research use only

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METHOD

Reagent for the determination of the Thrombin Time (TT) on plasma, using a clotting method, which can be manual, semi-automatic or automatic, using highly purified human Thrombin.

ASSAY PRINCIPLE

Measurement of the clotting time induced by thrombin, in presence of calcium, on plasma, and exploration of anti-thrombin activities.

SPECIMEN

Human plasma collected on citrate anticoagulant.

REAGENTS

6 vials of highly purified **human Thrombin**, stabilised and lyophilised in presence of calcium.

MATERIAL REQUIRED BUT NOT PROVIDED

- 100 µL Pipettes.
- Clotting instrument for semi-automatic or automatic coagulation assays, fibrometer or electromagnetic water bath.

PREPARATION, CONSERVATION AND STABILITY OF REAGENTS

When stored at 2-8°C in their original vials, and before any use, reagents are stable until the expiration date printed on the kit.

Preparation:

- **Calcium-Thrombin:** each vial must be restored with 2 ml of distilled water, in order to obtain a Calcium-Thrombin solution, containing a concentration of about 1.0 NIH/ml of Calcium-Thrombin*, ready to use.

This solution is stable, at least:

- 48 hours at room temperature
- 7 days at 2-8°C.

*The exact Thrombin concentration can vary from lot to lot and is adjusted for each lot in order to offer a high sensitivity Thrombin Time assay.

Note: Thrombin is prepared by activation of purified prothrombin extracted from human plasma. Plasmas used for prothrombin preparation were tested with registered methods and found negative for HIV antibodies, HBs Ag and HVC antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

ASSAY PROCEDURE

Specimen collection:

Blood (9 vol.) must be collected on 0.109M citrate anticoagulant (1 vol.); plasma supernatant is decanted following a 20 min. centrifugation at 2,500 g; citrated plasma should be tested within 4 hours or stored frozen at -20°C or below for up to 6 months, and thawed for 15 min. at 37°C just before use. Thawed specimen must be tested within 4 hours. EDTA collected human plasma may also be used.

Tested plasma:

Plasma must be tested undiluted.

Assay protocol:

Thrombin must be incubated at 37°C in its original vial or in a plastic tube.

In a test tube or in the reaction cuvette of the clotting instrument, introduce:

- 100 µl of citrated plasma
Incubate for 1 minute at 37°C, then introduce
- 100 µl of Calcium-Thrombin, starting the stopwatch
Note exactly the clotting time.

Résultats:

Thrombin time (TT) is abnormal if :
TT ≥ 25 seconds.

Usual values:

For normal plasmas Thrombin Time is usually in the range:
15 sec. to 22 sec.

Prolonged Thrombin Time

- A prolonged Thrombin Time (≥ 25 seconds) can result from:
 - Presence of antithrombin activity induced by therapy (Heparin, Hirudin).
 - Presence of high concentrations of Fibrin/Fibrinogen degradation products.
 - Qualitative (dysfibrinogenemia) or quantitative abnormalities of Fibrinogen (deficiency, DIC, fibrinolysis, hepatic disorders including cirrhosis).
- The Thrombin Time is normal in presence of a Factor XIII deficiency.

ASSAY CHARACTERISTICS

- Human Thrombin exhibits a higher efficiency than Bovine Thrombin for clotting Human Fibrinogen. Therefore, when the Thrombin Time is performed with Human Thrombin, that assay is less sensitive to trace amounts of heparin and Thrombin inhibitors than when it is realized using Bovine Thrombin. However, using Human Thrombin allows obtaining a high sensitivity to all direct Thrombin inhibitors (such as Hirudin or its derivatives, and new direct anti-Thrombin drugs) and the prolongation of clotting time is then proportional to the inhibitor concentration.
- HEMOCLOT THROMBIN (HUMAN) is prepared with highly purified Human Thrombin, mainly in the α-form, then fully biologically active. This reagent does not contain contaminants which can generate additional Thrombin from the tested plasma. It is then the reagent of choice for the exploration of all direct anti-Thrombin inhibitors, specifically targeted to Human Thrombin.

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