interchim

PENTAPHARM LTD

# Pefakit® in-TDT®

REF **800236**

**For research use only**

## Intended Use and Application

The Pefakit® in-TDT® (TDT, Thrombin Dynamics Test; in, intrinsic pathway) allows a kinetic determination of thrombin fn of the
intrinsic coagulation pathway. It is intended for research use only.

## Introduction

Thrombin is the key enzyme of hemostasis (1): it cleaves fibrinogen, is the most potent physiological platelet activator, activates factor XIII and causes several positive and negative feedback reactions (2). Changes in the concentration of coagulation factors, activators or inhibitors quantitatively determine the kinetics of thrombin generation (3-6). Routine methods for the functional assessment of the plasmatic coagulation factors, such as aPTT or prothrombin time (PT), detect the time point of free thrombin formation but do not quantify its kinetics (7). The latter are thought to be of fundamental importance for the hemostatic process in vivo (7). A stimulus leading to weak thrombin formation in vitro may not result in any free thrombin formation in vivo where flow and cellular interaction are present. Quantifying thrombin formation may also provide clinically relevant data for the management of patients with disease-related hypercoagulation or thrombophilia (8).

## Principle of the Method

Coagulation is triggered using a selected contact activator reagent. Thrombin formation is detected with a chromogenic substrate being readily converted by thrombin and releasing p-nitro-aniline (p-NA), which is continuously recorded at 405 nm (9). Fibrin gelation in the sample is inhibited by the addition of a fibrin polymerization inhibitor, hence allowing an undisturbed optical detection. The first derivative of the resulting curve is calculated, representing the velocity of the conversion of the chromogenic substrate. For test principle see also the cartoon in the appendix.

Two parameters are determined: the thrombin onset time delineates the time point when free thrombin is detected (begin of increasing optical density during the reaction). The second parameter is the peak value of the first derivative that represents the maximum velocity of conversion of the thrombin substrate and thereby characterizes the dynamics of thrombin formation. This parameter is defined as *thrombin dynamics* and is usually expressed as *% of normal*.

## Reagents

| Reagent | Content |
|---|---|
| **R1** | **in-TDT® Reagent**<br>(Pefachrom® TH, Pefabloc® Fg)<br>2 vials (lyophilisate, to be reconstituted in 2.0 ml in-TDT® Diluent (R2) per vial) |
| **R2** | **in-TDT® Diluent**<br>(25 mM $CaCl_2$)<br>2 vials, 2.5 ml (ready to use) |
| **R3** | **in-TDT® Activator**<br>(aPTT reagent based on ellagic acid)<br>2 vials, 2 ml (ready to use) |

Incubate reconstituted solutions R1 in the closed vial for 10 min at room temperature and swirl gently before use.

## Materials required but not provided

- Calibrated pipettes (1000 – 5000 µl)
- Automated or semi-automated coagulation instruments employing mechanical or optical detection methods

**Note:** When using automated or semi-automated coagulation analyzers refer always to manufacturer's operator manual or ask for a detailed adaptation protocol.

## Storage and Stability

The test kit may be used up to the expiry date given on the label when stored unopened at 2 – 8 °C.

Stability of the reagents after reconstitution or opening:

| Reagent | Stability | |
|---|---|---|
| **R1** | - 20 °C | 6 months |
| | 2 – 8 °C | 5 days |
| | 15 – 25 °C | 24 hours (on-board) |
| **R2** | - 20 °C | at least 2 years |
| | 2 – 8 °C | at least 18 months |
| | 15 – 25 °C | at least 3 months |
| **R3** | | do not freeze |
| | 2 – 8 °C | 30 days |
| | 15 – 25 °C | 24 hours (on-board) |

Frozen reagents should be thawed at room temperature and gently mixed before use. Freeze only once.

## Quality Control

Use Control N and P as references for validation of the assay. Control N and P are parts of the Pefakit® TDT® Calibrator and Controls [REF 800237]. A control run should be made with each test series. For preparation, use and certified ranges of the controls refer to the box insert instructions and certificate.

If values outside the specified range are obtained, a complete check of reagents should be made and the analysis should be repeated. If the problem persists, a complete instrument check should be made and the analysis should be repeated.

## Blood Collection and Sample Preparation

The patient should be at rest for 10 min prior sampling. Collect venous blood carefully in either 104 mM sodium citrate (volume ratio 9 + 1). Mix gently blood and anticoagulant directly after sampling, avoid foam formation. Centrifuge immediately at no less than 2000x g for at least 20 min at room temperature. Take care to avoid contaminations from the platelet layer into plasma when the plasma is separated from the cells. As a general rule hemolytic plasma samples should not be used.

For storage freeze undiluted plasma rapidly at - 70 °C in aliquots. Freeze only once. Avoid repeated freezing and thawing cycles. Thawing should be done rapidly (within 5 min) in a water bath at 37 °C. For more information see NCCLS document H21-A2 [10].

Stability of undiluted samples (plasma):

| | |
|---|---|
| - 80 °C | at least 1 year |
| - 20 °C | 2 months |
| 2 – 8 °C | 24 hours |
| 15 – 25 °C | 4 hours |

## Procedure

Prepare reagents as described above. Thaw frozen samples as described above ensuring negligible loss of activity of labile coagulation factors and absence of cryoprecipitate. Invert thawed sample for homogenization.

**Pipetting scheme:**

| | | Volume |
|---|---|---|
| | **Sample (platelet poor plasma) or control plasma** | 60 µl |
| **R3** | **in-TDT® Activator Reagent** **Dilution 1:3 in NaCl** (1 part reagent + 2 parts 0.9 % NaCl) | 60 µl |
| | **Incubation: 180 s, 37°C** | |
| **R1** | **in-TDT® Reagent** | 60 µl |

Start kinetic measurement at 405 nm. Monitor the rise in extinction over 400 s.

**Calculation of the first derivative:**

The first derivative of the optical density curve can be calculated as follows using standard computer software (e.g. MS Excel®):

First derivative at the time point x =
((OD at the time point x + 2 sec)
– (OD at the time point x - 2 sec)) / 4

**Calculation of the *thrombin dynamics*:**
The maximum velocity of the substrate conversion (maximum of the first derivative) is defined as the *thrombin dynamics*.

## Automated Procedure

Adaptation protocols for automated coagulation analyzers are available. Please contact Pentapharm.

## Calibration

Determine the thrombin dynamics of the calibrator. The calibrator is part of the Pefakit® TDT® Calibrator and Controls [REF 800237]. For preparation, use and certified value of the calibrator refer to the corresponding box insert instructions and certificate.

The thrombin dynamics of the samples is calculated as:

Thrombin Dynamics (sample) =
Maximum of first derivative (sample)
/ Maximum of first derivative (calibrator)
x certified value of the calibrator (% of normal)

## Expected Values

Typical ranges for normal healthy individuals on BCS are shown in the table below.

| Device | n | Thrombin dynamics (% of normal)* | Maximum of first derivative* |
|---|---|---|---|
| **BCS®** | 101 | 105 - 109 | 190 - 196 |

* 95% confidence range

## Specificity and Sensitivity

The Thrombin Dynamics Test is a global screening assay. The dynamics of initial thrombin formation can be modulated by any inhibitors or accelerators of the coagulation cascade dependent on the concentration or specific activity. Inhibitors such as heparinoids lead in a concentration dependent manner to a decrease of initial thrombin formation.

## Precision and Reproducibility

Intra-assay and inter-assay precision and reproducibility measurements were determined using Control N and P on two different instruments (BCS® and ACL9000). Control N and P are parts of the Pefakit® TDT® Calibrator and Controls [REF 800237]. Over these instruments and measurements the coefficient of variation (CV) within the series (n = 8 to 14) and between test series on 3 different days was ≤ 6 %. Looking at the series of thrombin dynamic measurements obtained with one sample on one instrument the CV was ≤ 6 %.

## Limitations and Interferences

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The assay only determines the initial thrombin activation phase. By the use of a chromogenic substrate which is readily converted by thrombin, the method has been optimized in order to provide a strong optical signal. However lipemic and hemolytic samples may interfere with the correct detection of the thrombin formation.

Being a global screening assay for determination of the dynamics of thrombin generation, the test will be influenced by all substances that interfere with the generation of thrombin, such as treatment with indirect or direct factor Xa inhibitors, inhibitors of factor IIa or coumarin therapy. Heparin spiked plasmas of different individuals show different dose response in their individual thrombin dynamics.

## Bibliography


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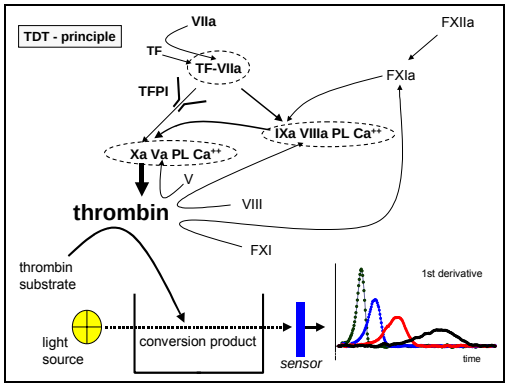
## Appendix





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