

TECHNOTHROMBIN[®] TGA

For research use only



GB

Complete reagent kit:

REF 5006010 TECHNOTHROMBIN[®] TGA Kit








Modular reagents:

REF 5006209	TECHNOTHROMBIN [®] TGA RB	5 x 0.5 mL
REF 5006210	TECHNOTHROMBIN [®] TGA RB	50 x 0.5 mL
REF 5006212	TECHNOTHROMBIN [®] TGA RC Low	5 x 0.5 mL
REF 5006213	TECHNOTHROMBIN [®] TGA RC Low	50 x 0.5 mL
REF 5006214	TECHNOTHROMBIN [®] TGA RC High	5 x 0.5 mL
REF 5006216	TECHNOTHROMBIN [®] TGA RC High	50 x 0.5 mL
REF 5006220	TECHNOTHROMBIN [®] TGA RD	5 x 0.5 mL
REF 5006222	TECHNOTHROMBIN [®] TGA RD	50 x 0.5 mL
REF 5006235	TECHNOTHROMBIN [®] TGA SUB	5 x 1.5 mL
REF 5006230	TECHNOTHROMBIN [®] TGA SUB	50 x 1.5 mL

Controls and Calibrator:

REF 5006310	TECHNOTHROMBIN [®] TGA C1	5 x 1 mL
REF 5006320	TECHNOTHROMBIN [®] TGA C2	5 x 1 mL
REF 5006330	TECHNOTHROMBIN [®] TGA C3	5 x 1 mL
REF 5006345	TECHNOTHROMBIN [®] TGA CAL Set	1 Set

Symbols key

Symbols key					
	Manufactured by		determinations	DIL	dilute or dissolve in
	expiry date	AQUA	Distilled water	LOT	lot
	Storage Temperature	BUF	buffer	REF	Catalogue number
	Consult Instructions for use	CAL	calibrator	RUO	research use only
CE	CE Mark	CONT	control	SUB	substrate

PRODUCT DESCRIPTION

INTENDED USE

The TECHNOTHROMBIN® TGA kit is an assay system for determination of thrombin generation over time in **platelet poor or platelet rich plasma** (PPP or PRP) upon activation of the clotting cascade by micelles of negatively charged phospholipids containing different amounts of human tissue factor and CaCl₂. The kit can be used to monitor hemophiliacs during inhibitor bypassing therapy, to monitor anticoagulation therapy, to calculate INR values for patients and to determine states of bleeding disorders or thrombophilia as well as the activity of circulating micro particles. This broad range of applications is possible by providing different tissue factor concentrations and by monitoring the whole kinetic of thrombin generation during initiation, amplification and down regulation of thrombin formation. TECHNOTHROMBIN® TGA is therefore a universal assay kit for analyzing and monitoring the function of the haemostatic system.

TEST PRINCIPLE

TECHNOTHROMBIN® TGA is based on monitoring the fluorescence generated by the cleavage of a fluorogenic substrate by thrombin over time upon activation of the coagulation cascade by different concentrations of tissue factor and negatively charged phospholipids in plasma. From the changes in fluorescence over time, the concentration of thrombin (nM) in the sample can be calculated using the respective thrombin calibration curve. The increase in thrombin concentration with time then allows to calculate generation of thrombin in the sample and to plot such thrombin values over time for the whole coagulation process. This then results in the visualization of the different phases of clot formation.

COMPOSITION

The TECHNOTHROMBIN® TGA reagent kit for 3x16 determinations contains:

mL	reagent	description
3 x 1.5	TGA substrate (SUB)	Fluorogenic substrate 1 mM Z-G-G-R-AMC, 15 mM CaCl ₂
1 x 3	TGA buffer (BUF)	Hepes-NaCl-buffer containing 0.5 % bovine serum albumin
1 x 0.5	TGA thrombin calibrator	~1.000 nM thrombin in buffer with BSA
1 x 0.5	TGA reagent B (RB)	Low conc. of phospholipid micelles containing ~ 2 pM rhTF in Tris-Hepes-NaCl buffer
1 x 0.5	TGA reagent C (RC) Low	Low conc. of phospholipid micelles containing ~ 5 pM rhTF in Tris-Hepes-NaCl buffer
1 x 0.5	TGA reagent C (RC) High	High conc. of phospholipid micelles containing ~ 5 pM rhTF in Tris-Hepes-NaCl buffer
1 x 1	TGA control 1 (C1)	Normal human plasma, lyophilized.
1 x 1	TGA control 2 (C2)	Human plasma with increased thrombin generation, lyophilized.
1 x 1	TGA control 3 (C3)	Human plasma with decreased thrombin generation, lyophilized.

Each reagent is available on a modular basis.

The TECHNOTHROMBIN® TGA RD contains:

mL	reagent	description
5 x 0.5 50x0.5	TGA reagent D	High conc. of phospholipid micelles containing ~ 200 pM rhTF in Tris-Hepes-NaCl buffer

The TECHNOTHROMBIN® TGA CAL Set contains:

mL	reagent	description
1 x 3	TGA buffer (BUF)	Hepes-NaCl-buffer containing 0,5 % bovine serum albumin
1 x 0.5	TGA thrombin calibrator	~1.000 nM thrombin in buffer with BSA

MATERIAL REQUIRED (not supplied with the kit)

- Pipettes
- Distilled water
- Microtiter plates suitable for fluorescence measurement (we recommend black NUNC Maxisorp REF 475515)
- Fluorimeter, fluorescence reader (96-well format), ~360 nm/~460 nm (excitation/emission) with suitable software to monitor changes of fluorescence over time. Applications for several readers are available as download from www.technoclone.com.

WARNING AND PRECAUTIONS

- for research use only
- Every single donor plasma and every lot of the controls included is tested and found negative for Hb_sAg, HIV 1/2 antibodies and HCV antibodies. However, general precautions should be taken by handling all human source materials as potentially infectious.
- All blood and plasma samples and products have to be handled as potentially infectious and with appropriate care and in compliance with the respective biosafety regulations and must be disposed in the same way as hospital waste.

STABILITY AND STORAGE

The expiry date printed on the labels is only applicable to storage of the unopened containers at + 2...8 °C.

Stability after reconstitution:

Reagent	RT* (20...25°C)	+2...8°C	-20°C
TGA substrate (SUB)	1 week	1 month	6 months
TGA buffer (BUF)	8 hours	1 week	1 month
TGA thrombin calibrator (CAL)	8 hours	1 week	6 months
TGA reagent B (RB)	8 hours	1 week	6 months
TGA reagent C (RC) Low and High	8 hours	1 week	6 months
TGA reagent D (RD)	8 hours	1 week	6 months
TGA control 1 (C1)	4 hours	8 hours	1 month
TGA control 2 (C2)	4 hours	8 hours	1 month
TGA control 3 (C3)	4 hours	8 hours	1 month

Avoid contamination by micro-organisms.

Plasmas should be frozen only once; during storage, the vials should be tightly capped.

Stability of the sample material:

* room temperature

Sample material	RT* (20...25°C)	+2...8°C	-20°C
PPP, PRP and PFP Plasma	2 hours	4 hours	1 month

An immediate centrifugation after blood withdrawal is recommended.

Further we recommend an immediate shock freezing of the centrifuged samples.

Attention! The frozen samples should be stored in a constant environment - avoid exposing the samples to variations in temperature.

Before transportation we recommend to centrifuge and prepare the samples.

TEST PROCEDURE

PREPARATION OF SAMPLES

In the TECHNOTHROMBIN® TGA assay citrated plasma (platelet rich, platelet poor or platelet free) can be used, depending on the specific application.

For plasma separation, mix 9 parts of venous blood and 1 part sodium citrate solution (0.11 mol/L) and centrifuge for 15 minutes at a RCF of at least 2.500 x g (corresponding to DIN 58905).

For special requirements, preparation of other plasmas might be necessary:

- for *platelet rich plasma (PRP)* centrifuge for 5 minutes at 100 x g and carefully pipette off the obtained PRP;
- for *platelet poor plasma (PPP)* centrifuge PRP for 10 minutes at 1.500 x g and carefully pipette off the obtained PPP;
- for *platelet and micro particle free plasma (PFP)*, centrifuge PPP for 30 minutes at 15.000 x g and carefully pipette off the obtained PFP or use the Technoclone micro particle filtration unit.

PREPARATION OF REAGENTS

The lyophilized reagents must be dissolved in the volume of distilled water indicated on the vials. All reconstituted reagents should **reach room temperature before use**.

After exactly 20 minutes of reconstitution time and thorough mixing (Vortex), reagents are ready to use.

For standardization tests a reconstitution time of 30 minutes is recommended for controls.

READER SETTING

Please use the corresponding **reader application** (provided under www.technoclone.com).

Temperature during measurement: **37°C**

Fluorometer **wavelength**: ~360 nm / ~460 nm [excitation/emission]

Attention !

A pre-reading of the empty plate is suggested, to avoid any inaccuracies during the reading of your samples, which can occur due to inhomogeneous and defective plates.

READING TIMES

1.) Thrombin calibration curve: 10 min

in 30 sec measurement intervals

The thrombin calibration curve has to be done separately from sample measurement.

2.) Samples: depending on the sample material 60 min (for FVIII inhibitor therapy 90 - 120 min) in 1 min measurement intervals.

PERFORMANCE OF THE TEST

Samples and dissolved reagents should reach room temperature before use.

1.) Thrombin calibration curve

The thrombin calibration curve has to be done separately from sample measurement. Concentration of the thrombin calibrator (CAL) is lot dependent, consult the label on the vial.

The thrombin calibrator is diluted with TGA buffer as indicated in the table below:

1st dilution (1:2): (STD 1)	+ 200 µL Thrombin Calibrator (CAL) 200 µL TGA buffer (BUF)
2nd dilution (1:4): (STD 2)	+ 100 µL 1 st dilution 100 µL TGA buffer (BUF)
3rd dilution (1:20): (STD 3)	+ 20 µL Thrombin Calibrator (CAL) 380 µL TGA buffer (BUF)
4th dilution (1:200): (STD 4)	+ 20 µL 3 rd dilution 180 µL TGA buffer (BUF)

All calibrator dilutions have to be **measured in duplicate**.

Add reagents in the following sequence:

40 µL	calibrator dilution (STD 1 - STD 4)
50 µL	TGA substrate (SUB)
measure for 10 min in 30 sec intervals at 37°C	

Start reading of the plate/strip immediately after pipetting the substrate.

ONLY ONE CALIBRATION CURVE HAS TO BE DONE FOR EACH LOT !

2.) Sample measurement

The reagents have to be added in the following sequence:

Reagent	Measurement with:			
	TGA RB	TGA RC Low	TGA RC High	TGA RD
sample	40 µL	40 µL	40 µL	40 µL
TGA RB	10 µL	-	-	-
TGA RC Low	-	10 µL	-	-
TGA RC High	-	-	10 µL	-
TGA RD	-	-	-	10 µL
TGA SUB	50 µL	50 µL	50 µL	50 µL
measure for 60 min (for FVIII inhibitor therapy 90 - 120 min) in 1 minute measurement intervals at 37°C				

Start reading of the plate immediately after pipetting the substrate.

A **reagent substrate mixture** can be prepared in advance.

Preparation of the mixture:

The mixture of reagent and substrate should be done in a **1+5 proportion**.

(Example: 200 µL Reagent + 1000 µL Substrate)

The mixture can be aliquoted and frozen at -20°C.

When reagent/substrate mixture is used the reagents have to be added to the plate in the following sequence:

Reagent	Measurement with reagent/substrate mixture:			
	TGA RB	TGA RC Low	TGA RC High	TGA RD
sample	40 µL	40 µL	40 µL	40 µL
reagent/substrate mixture	60 µL	60 µL	60 µL	60 µL
measure for 60 min (for FVIII inhibitor therapy 90 - 120 min) in 1 minute measurement intervals at 37°C				

Start reading of the plate immediately after pipetting the reagent/substrate mixture.

Attention ! We recommend to measure duplicates for each samples.

ANALYSIS OF RESULTS

Evaluation is done automatically with the TECHNOTHROMBIN® TGA evaluation software (Software for several readers are available as download from www.technoclone.com). The software includes calibration curve and sample evaluation.

THROMBIN CALIBRATION CURVE

Using the provided evaluation software, RFU data (relative fluorescence units) measured by the fluorimeter for the different thrombin concentrations are converted into a thrombin calibration curve. This thrombin calibration curve is then used by the provided software to calculate nM thrombin present in the sample at a given time.

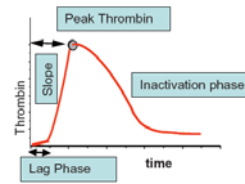
STANDARDIZATION

The thrombin calibrator is calibrated against the thrombin Reference Preparation of the WHO.

ANALYSIS OF SAMPLES

The provided evaluation software (www.technoclone.com) calculates thrombin generation in the sample over time and the results are given in nM

thrombin generated in the sample for each point of time during the whole coagulation process. Upon initiation of coagulation in the samples by addition of CaCl₂ and the phospholipid/tissue factor mixture, generation of thrombin is initiated after a lag period; thereafter thrombin generation per minute increases, reaching a maximum of thrombin generated and decreases thereafter. The pattern seen resembles the figure provided below:



The following parameters can be used as readout in our software:

- Lag phase** from the time point when the TGA reagent including CaCl₂ is added until the first burst in thrombin formation
- Slope**: Steepest rate of thrombin formation per minute.

Calculated by the software as velocity index

$$\text{Velocity Index} = \frac{\text{peak thrombin}}{\text{peak time} - \text{lag time}}$$

3. **Peak thrombin**: Maximal concentration of thrombin formed

4. **AUC**: Area under the curve

Slope and peak thrombin can depend on the amount of phospholipids present in the sample. Since the provided amount of phospholipids in reagents RB and RC Low is limited this value is determined in PPP by the number and composition of micro particles present in the sample. In most instances there is a good correlation between slope and peak thrombin. Both parameters also depend on the amplification of initial thrombin generated and are higher in states of thrombophilia and decreased during anticoagulation therapy or in patients with bleeding disorders.

We recommend the following applications:

Reagent	Purpose
TGA RB	- to monitor inhibitor bypass therapy with FEIBA in hemophiliacs with Factor VIII inhibitors
TGA RC Low	- measurement of thrombophilia tendency (preferentially in standard PPP plasma) - measurement of bleeding tendency - to monitor inhibitor bypass therapy with rFVIIa in hemophiliacs with Factor VIII inhibitors
TGA RC High	- monitoring anticoagulation therapy
TGA RD	- monitoring heparin anticoagulation, direct thrombin and Xa inhibitor therapy

NORMAL RANGE

The expected values for PPP and PRP plasmas are:

Sample	Reagent	Peak Thrombin nM	SD (Standard deviation)
PPP (Platelet Poor Plasma)	RB	311.7	149.2
	RC Low	289.5	151.9
	RC High	597.0	225.7
PRP (Platelet Rich Plasma)	RB	593.3	108.9
	RC Low	602.2	106.9
	RC High	733.4	102.8

REFERENCE RANGE

In a randomly selected population of "healthy" individuals the range of peak thrombin and the thrombin slope was comparable to the values obtained in the normal control plasma provided in the kit. Anticoagulated patients with an INR around 2.0 have average peak thrombin and thrombin slopes as the anticoagulation control provided. Patients with e.g. homozygous protein C deficiency present themselves with values comparable to the thrombophilia control provided in the kit. It is important to note that the controls included in the kit contain an average amount of circulating micro particles also found in a normal population and cannot be used as PFP controls. Micro particle free normal controls can be purchased separately.

Please consult the lot specific batch table included in the kit for the reference ranges.

LIMITATION OF THE TEST

Reliable results can only be obtained when blood collection is standardized and follows the criteria of minimal activation of the clotting system during venipuncture. Care has to be taken during centrifugation of blood and plasma that only such plasma samples are used for the assays that comply with the requirements for the respective assays. In case of use of incorrect plasma samples interpretation of the results might become impossible. Inaccurate results can occur due to inhomogeneous and defective plate, inaccurate pipetting and delayed readings after pipetting.

LITERATURE

For literature please consult our website www.technoclone.com.