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TECHNOTHROMBIN® TGA RA/MFC

For research use only

GB

REF 5006205	TECHNOTHROMBIN® TGA RA	5 x 0.5 mL
REF 5006206	TECHNOTHROMBIN® TGA RA	50 x 0.5 mL
Microparticle free Control:		
REF 5006350	TECHNOTHROMBIN® TGA MFC (microparticle free control)	5 x 0.5 mL

Symbols key / Symbolschlüssel					
	manufacturer / Hersteller	**AQUA**	Distilled water / destilliertes Wasser	**MFC**	microparticle free control / Micropartikel freie Kontrolle
	expiry date / Verfallsdatum	**BUF**	buffer / Puffer	**REF**	Catalogue number / Katalognummer
	Storage Temperature / Lagertemperatur	**CAL**	calibrator / Kalibrator	**RUO**	research use only / nur für Forschungszwecke
	Consult Instructions for use / Gebrauchsanweisung beachten	**CONT**	control / Kontrolle	**SUB**	substrate / Substrat
	CE Mark / CE-Zeichen	**DIL**	dilute or disolve in / verdünnen oder lösen in		
	determinations/ Bestimmungen	**LOT**	lot / Charge		

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TECHNOTHROMBIN® TGA RA/MFC

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PRODUCT DESCRIPTION

INTENDED USE

The TECHNOTHROMBIN® TGA RA/MFC are reagents to monitor the activity of circulating microparticles contained in platelet poor plasma (PPP) for use together with TECHNOTHROMBIN® TGA reagents (see below). TECHNOTHROMBIN® TGA Reagent A (RA) does not contain tissue factor and thrombin generation is only initiated by the contained micelles of negatively charged phospholipids.

TEST PRINCIPLE

Thrombin generation over time can be determined by TECHNOTHROMBIN® TGA. This assay is based on monitoring the fluorescence generated by the cleavage of a fluorogenic substrate by thrombin over time upon activation of the coagulation cascade. 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. When the coagulation cascade is initiated by TECHNOTHROMBIN® TGA Reagent A (RA) the lag phase (see below) and the maximal thrombin generation (see below) are dependent on the number and activity of circulating microparticles contained in the sample. The difference in thrombin generation measured in PPP and microparticle free plasma (MPFP) even better reflects the microparticle content of the sample.

COMPOSITION

The TECHNOTHROMBIN® TGA reagent A (RA) is available in two package sizes REF 5006205 for 5x0.5 mL or REF 5006206 50x0.5 mL of TGA Reagent A:

mL	reagent	description
0.5	TGA reagent A (RA)	Low conc. of phospholipid micelles in Tris-Hepes-NaCl buffer

The TECHNOTHROMBIN® TGA microparticle free control (MFC) REF 5006350 contains:

mL	reagent	description
5 x 0.5	TGA microparticle free control (MFC)	obtained from specifically prepared platelet poor plasma by centrifugation and filtration

MATERIAL REQUIRED (not supplied with the kit)

- Pipettes
- Distilled water
- Additional reagents

REF	5006235	TECHNOTHROMBIN® TGA SUB	5 x 1.5 mL
REF	5006230	TECHNOTHROMBIN® TGA SUB	50 x 1.5 mL

- Additional controls and calibrators

REF	5006320	TECHNOTHROMBIN® TGA CH	5 x 1 mL
REF	5006330	TECHNOTHROMBIN® TGA CL	5 x 1 mL
REF	5006345	TECHNOTHROMBIN® TGA CAL Set	1 Set

- 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 from sales@technoclone.com.

WARNING AND PRECAUTIONS

- 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 reagent A (RA)	8 hours	1 week	6 months
TGA microparticle free control (MFC)	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

For determination of circulating microparticles In the TECHNOTHROMBIN® TGA assay citrated plasma (platelet poor and microparticle free) should be used.
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). Standard PPP is obtained that can be further processed to MPFP. However, during centrifugation ex vivo formation of microparticles is possible.

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.

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 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.

READER SETTING

Please use the corresponding **reader application** (provided on requested, please contact sales@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
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 1st 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 3rd 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:

	Measurement with:
Reagent	TGA RA
sample TGA RA TGA Substrate	40 µL 10 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:

	Measurement with reagent/substrate mixture:
Reagent	TGA RA
sample reagent/substrate mixture	40 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 THE RESULTS

Evaluation is done automatically with the TECHNOTHROMBIN® TGA evaluation software (provided on request, please contact sales@technoclone.com). The software includes calibration curve and sample evaluation.

THROMBIN CALIBRATION CURVE

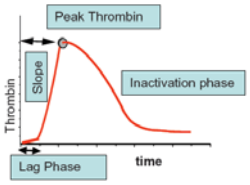
Using the provided evaluation software (can be requested from sales@technoclone.com), RFU data (relative fluorescence units) measured by the fluorimeter for the different thrombin concentrations are converted into a thrombin calibration curve. This thrombin calibration curve is then used by the provided 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 software (can be requested from sales@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 clotting in the samples by addition of $CaCl_2$ and the phospholipid 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:

1. **Lag phase** from the time point when the TGA reagent including $CaCl_2$ is added until the first burst in thrombin formation
2. **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

The lag phase depends ion the amount of tissue factor contained in the circulating microparticles in the plasma samples
Slope and peak thrombin depends on the amount of phospholipids present in the sample. Since the provided amount of phospholipids in reagents RA 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.

NORMAL RANGE

The expected values for PPP and MPF plasma are:

Sample	**Reagent**	**Peak Thrombin nM**	**SD (Standard deviation)**
PPP (Platelet Poor Plasma)	RA	276.2	104.8
MPFP (Micro Particle free Plasma)	RA	No significant thrombin generation should be obtained	

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.