

TECHNOTHROMBIN® TGA

SHORT INSTRUCTION

READER SETTINGS:

Please use the corresponding **reader application**, which can be requested from info@diapharma.com.

Temperature during measurement: **37°C**

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**.

The results from calibration curve and sample measurement will be brought together in the Excel evaluation software for TECHNOTHROMBIN® TGA provided by DiaPharma or Technoclone via email.

1. THROMBIN CALIBRATION CURVE

The lyophilized reagents have to be **dissolved in the volume of distilled water indicated in the table below and on the vials**. The concentration of the thrombin calibrator [CAL] is lot dependent, consult the label on the vial.

Reagents	Dissolve in
TGA thrombin calibrator [CAL]	0.5 mL
TGA buffer [BUF]	3 mL
TGA substrate [SUB]	1.5 mL

All dissolved reagents should reach **room temperature** before use.
After 20 minutes of reconstitution time and thorough mixing (Vortex) reagents are ready to use.

Dilution of the calibrator:

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]

Measurement of Calibration Curve:

Please use microtiter plates suitable for fluorescence measurement (we recommend NUNC Maxisorp 475515)
All calibrator dilutions have to be **measured in duplicate**.

Plate Layout Calibration curve:

	1	2	3	4	5	6
A	STD1					
B	STD1					
C	STD2					
D	STD2					
E	STD3					
F	STD3					
G	STD4					
H	STD4					

To obtain the **calibration curve** add reagents in the following sequence:

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

Start reading of the plate immediately after pipetting the substrate.

The reader should have reached 37 °C before measurement !

IMPORTANT: Pipette the dilutions of the calibrator according to the plate layout (from A1 - H1).

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

2. SAMPLE MEASUREMENT

The lyophilized reagents have to be **dissolved in the volume of distilled water indicated in the table below** and on the vials.

Reagents	Dissolve in
TGA substrate SUB	1.5 mL
TGA reagent A RA	0.5 mL
TGA reagent B RB	0.5 mL
TGA reagent C Low RC Low	0.5 mL
TGA reagent C High RC High	0.5 mL
TGA control C1 C1	1 mL
TGA control C2 C2	1 mL
TGA control C3 C3	1 mL

All dissolved reagents should reach **room temperature** before use.
After 20 minutes of reconstitution time and thorough mixing (Vortex) reagents are ready to use.

The reagents have to be added to the plate in the following sequence:

Reagent	Measurement with:			
	TGA RA	TGA RB	TGA RC Low	TGA RC High
sample/control	40 µL	40 µL	40 µL	40 µL
TGA RA	10 µL	-	-	-
TGA RB	-	10 µL	-	-
TGA RC Low	-	-	10 µL	-
TGA RC High	-	-	-	10 µL
TGA Substrate	50 µL	50 µL	50 µL	50 µL

measure for 60 min (for FVIII inhibitor therapy 90 - 120 min) in 1 min measurement intervals

Start reading of the plate immediately after pipetting the substrate.

As an alternative 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.

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 RA	TGA RB	TGA RC Low	TGA RC High
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 min measurement intervals

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

Example plate layout for measurement of duplicates and usage of 3 different TGA reagents and controls.

Reagent	RB	RB	RC low	RC low	RC high	RC high
	1	2	3	4	5	6
A	C1	C1	C1	C1	C1	C1
B	C2	C2	C2	C2	C2	C2
C	C3	C3	C3	C3	C3	C3
D	SPL1	SPL1	SPL1	SPL1	SPL1	SPL1
E	SPL2	SPL2	SPL2	SPL2	SPL2	SPL2
F	SPL3	SPL3	SPL3	SPL3	SPL3	SPL3
G	SPL4	SPL4	SPL4	SPL4	SPL4	SPL4

IMPORTANT:

The software recognizes duplicates of samples only when they are pipetted next to each other (not one below each other as for the calibration curve) according to the plate layout.

The reader should have reached 37 °C before measurement !