For Research Use Only

September/2021

SMAT – TF kit

Assay for Determination of Tissue Factor - Driven Thrombin Generation

The kit is for research purpose only, not for in vitro diagnostics

INTENDED USE

The smart analysis of thrombin production (SMAT) - TF is the assay system with high sensitivity for determination of thrombin generation (TG) in plasma in an early phase of coagulation initiated by the tissue factor (TF) pathway. The kit can be used to monitor the anticoagulation drugs such as direct oral anticoagulants and the bypassing agents for hemophilia patients. The kit is also intended to evaluate abnormality in blood coagulation in patients with thrombotic and hemorrhagic disorders and to investigate association of cancer and infectious diseases such as COVID-19 with thrombosis.

ASSAY PRINCIPLE

The SMAT - TF assay provides an approach for detecting extremely low concentrations of thrombin (FIIa) generated in plasma prior to a FIIa burst. To determine the TF-driven TG, the TF initiator reagent including re-lipidated TF, phospholipids, and anti-FVIIIa inhibitory antibody is incubated with citrated plasma in the presence of calcium ion to induce TG. Ethylenediaminetetraacetic acid (EDTA) solution terminates TG and then fluorescence generated by FIIa cleavage of fluorogenic substrate is kinetically measured. The TG is expressed as a relative ratio (%) to that of human control plasma. Of note, the assay is completed within 10 min.

MATERIALS

Materials Supplied in the Kit

The kit for 20 determinations contains 6 reagents (2 with lyophilization and 4 in solution) in microtubes with different color caps for identification:

Kit Components

Cap colors	Tubes	Reagents with Lyophilization
White	1	TF Initiator
Red	1	Human Control Plasma
Cap colors	**Volume**	**Reagents in Solution**
Yellow	1 × 1 mL	$CaCl_2$
Green	1 × 0.15 mL	Thrombin Calibrator (4,000 pM)
Blue	1 × 1 mL	Reconstitution Buffer
Brown	1 × 1.4 mL	Fluorogenic FIIa substrate/EDTA

Materials Required – Not Supplied in the Kit

- Fluorescent plate reader (~360 nm excitation/~460 nm emission) with temperature control at 37°C and suitable software capable to monitor changes of fluorescence intensity over time
- Single - and repeater pipettes
- 96-well microtiter plate (flat bottom)

Preparation of Blood and Plasma Samples

Blood samples are prepared by carefully mixing 9 parts venous blood with 1 part 3.2% sodium citrate solution. Platelet-poor plasma samples are prepared by centrifugation of blood at 25°C for 15 min at 2,000 × g. Immediately after centrifugation, plasma is frozen and stored below -70°C.

PRECAUTIONS FOR USE OR HANDLING

- For research use only
- Control plasma included in the kit is tested and found negative for Hb_SAg, HIV 1/2 antibodies and HCV antibodies, but control plasma and plasma samples have to be handled as potentially infectious materials with appropriate care and in compliance with the respective biosafety regulations.
- All materials contaminated with blood or plasma must be disposed as biohazardous waste.

STORAGE

The kit should be stored at 4-8°C before use. The expiry date printed on the box label is only applicable to storage of the kit. The reconstituted initiator reagent can be stored at 4-8°C and used within 4 days.

ASSAY PROCEDURE

Preparation of Reagents for TG Assay

- Preparation of the TF initiator reagent solution: The TF initiator reagent solution (white cap) is prepared by adding **0.32 mL** of reconstitution buffer (blue cap) and then mixing (vortex). The reagent solution can remain at ~25°C.
- Reconstitution of control plasma: Control plasma (red cap) is reconstituted with **0.1 mL** of distilled water for 5 min at ~25°C. Plasma can remain at ~25°C for a maximum for 4 h.
- The reagents in solution supplied in the kit: The reagents stored at 4-8°C should be warmed to ~25°C before use. The $CaCl_2$ solution (yellow cap) and FIIa substrate/EDTA (brown cap) are kept at 37°C prior to the assay.

Plate Reader Setting

- Temperature during assay procedures and fluorescence measurement: 37°C
- Fluorometer wavelength: ~360 nm/~460 nm (excitation/emission)

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PERFORMANCE OF THE TG ASSAY

The TG assay is carried out according to the following workflow. To create a workflow program using the plate reader operation software is strongly recommended for more accurate and consistent results.

1. Workflow for TG Assay with Control Plasma and Plasma Sample

(1) Add 35 µL of control plasma (red cap) and plasma sample to each 96-well microtiter plate wells.
(2) Insert plate into plate reader to incubate plate at 37°C for 2 min.
(3) Remove plate from plate reader.
(4) For each plasma, use a repeater pipette to add 10 µL the TF initiator reagent solution (white cap) to a plate well.
(5) Insert plate into plate reader to incubate plate at 37°C for 2 min.
(6) Remove plate from plate reader.
(7) Add 10 µL $CaCl_2$ solution (yellow cap) to each plate well utilizing repeater pipette.
(8) Insert plate into plate reader to incubate plate at 37°C for 2.5 min.
(9) Remove plate from plate reader.
(10) Add 50 µL FIIa substrate/EDTA solution (brown cap) to each plate well utilizing repeater pipette.
(11) Insert plate into 37°C plate reader. Measure fluorescence intensity generated by FIIa cleavage of the fluorogenic substrate at 37°C for ~1 min in ~10 sec intervals.
(12) Monitor relative fluorescence units (RFU) over time and plot data with plate reader operation software.

Attention!

Plasma sample should be tested simultaneously with control plasma.

2. Workflow for Assay with the FIIa Calibrator

Prior to testing plasma, assay of the FIIa calibrator activity (green cap) is recommended to verify that the fluorescence measurement with plate reader and workflow program works correctly. The assay is performed in the same way as in TG with plasma, but the steps (4) and (7) for testing plasma are skipped.

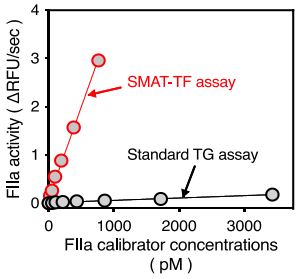
(1) Add 55 µL of the FIIa calibrator and reconstitution buffer (blue cap) to each plate wells.
(2) Insert plate into plate reader to incubate plate at 37°C for 2 min.
(3) Remove plate from plate reader.
(4) Insert plate into plate reader to incubate plate at 37°C for 2 min.
(5) Remove plate from plate reader.
(6) Insert plate into plate reader to incubate plate at 37°C for 2.5 min.
(7) Remove plate from plate reader.
(8) Add 50 µL FIIa substrate/EDTA solution (brown cap) to each plate well utilizing repeater pipette.
(9) Insert plate into 37°C plate reader. Measure fluorescence intensity generated by FIIa cleavage of the fluorogenic substrate at 37°C for ~1 min in ~10 sec intervals.
(10) Monitor relative fluorescence units (RFU) over time and plot data with plate reader operation software.

EVALUATION OF THE TG IN PLASMA SAMPLE

Based on plotted RFU data over time, a mean value of slope (ΔRFU/second) is calculated by a linear regression analysis of data with plate reader operation software or Excel (after data are exported). Slope value is equal to the levels of FIIa generated in plasma. The TF-driven TG is evaluated by dividing the slope value of plasm sample by that of control plasma. The result is expressed as a relative ratio (%) to control plasma: TG (%) = (the slope value of plasma sample ÷ the slope value of control plasma) × 100. The TG parameter depends on initial thrombin generated.

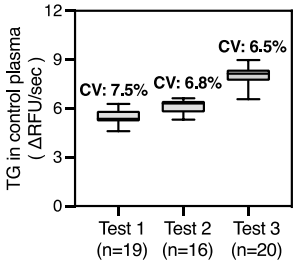
ASSAY SENSITIVITY AND PRECISION

1. Assay Sensitivity



Compared to standard TG assay, the SMAT-TF assay has higher sensitivity for detection of the FIIa activity.

2. Precision



Testing intra-assay variation in TG assay of control plasma indicates high reproducibility.

REFERENCE

(1) Selective factor VIII activation by the tissue factor-factor VIIa-factor Xa complex. Blood 130: 1661-1670, 2017

CONTACT

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