IMMBIOMED

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TAFI Activity Assay

Product no. ADG871

Storage: 2 – 8°C For Research Use Only!

PRODUCT INSERT ENGLISH

INTENDED USE

The TAFI Activity assay is a plasma based chromogenic assay for the determination of **T**hrombin **A**ctivatable **F**ibrinolysis **I**nhibitor (TAFI) enzyme activity.

INTRODUCTION

TAFI (**T**hrombin **A**ctivatable **F**ibrinolysis **I**nhibitor) is a proenzyme similar to carboxypeptidase B and is activated to TAFIa by thrombin and probably plasmin [1,2]. Thrombomodulin accelerates TAFI activation about 1250 times. TAFIa is an important inhibitor of fibrinolysis by cleaving C-terminal lysine and arginine residues in the fibrin clot. Thereby the carboxy-terminal plasminogen binding sites in the fibrin clot are degraded, plasminogen activation by t-PA and in consequence fibrinolysis is most effectively inhibited [3,4,5,6]. High levels of TAFIa may be an indication for thrombotic risk [7,8,9,10]. Properties of TAFI:

- Plasma concentration: 4–15 µg/ml
- Molecular weight: 55 kDa (401 aa, glycoprotein containing zinc)
- Conversion to TAFIa: by cleavage at Arg-92 to a 36 kDa peptide
- Mode of action of TAFIa: basic carboxypeptidase, cleaves C-terminal lysine and more specifi cally arginine residues. Inhibition of fibrinolysis by removal of plasminogen binding sites in the fibrin clot.
- Stability of TAFIa: from several hours at 22 °C to 10 min at 37 °C by conformational instability.

PRINCIPLE OF THE METHOD

The synthetic substrate is a substituted peptide mimetic consisting of an amino-protected L-lysine connected with an L-arginine of which the α-position of the side chain is a sulphur atom. It is degraded selectively and irreversibly by TAFIa producing a thiol derivative. This thiol reacts chemically with the colourless Ellman's reagent (5,5'-Dithio-bis-(2-nitrobenzoic acid), DTNB) splitting off the yellow coloured 5-mercapto-2-nitro-benzoic acid. The extinction measurable at the wavelength of 405 nm at the end of the enzymatic reaction is directly proportional to the concentration of TAFI activated by thrombin/thrombomodulin.

REAGENTS, PREPARATION AND USE

Reagent	Content
R1	**Activator** 2 vials, lyophilised (to be reconstituted in 4.0 ml of deionized water)
R2	**Start Reagent** 2 vials; lyophilised (to be reconstituted in 4.0 ml of Diluent R3)
R3	**Diluent** 2 vials, ready to use (diluent for reconstitution of the Start Reagent R2)
CAL	**TAFI Calibrator** (human plasma) 1 vial, lyophilised (to be reconstituted in 1.0 ml of deionized water)
C1	**TAFI Control 1** (human plasma) 1 vial, lyophilised (to be reconstituted in 1.0 ml of deionized water)
C2	**TAFI Control 2** (human plasma) 1 vial, lyophilised (to be reconstituted in 1.0 ml of deionized water

Incubate reconstituted solutions R1 and R2 in closed vials for 10 min at room temperature and swirl gently before use.

PRECAUTIONS

Each donor unit used in the preparation of human source reagent has been tested for antibodies against HIV Type 1 and 2, Hepatitis C-Virus, Treponema pallidum as well as Hepatitis B surface-antigen and Hepatitis C-genome by PCR. The plasmas were found to be negative on the tested parameters. However, since no test can completely rule out the presence of blood borne diseases these control plasmas have to be handled as potentially infectious material.

MATERIALS REQUIRED BUT NOT PROVIDED

0.9% sodium chloride
Calibrated pipettes (10–5000 µl)
Microtiter plates
Microtiter plate reader or automated or semi-automated coagulation instruments which employ an optical detection channel.

Note: When using automated or semi-automated coagulation analyzers refer always to manufacturer's operator manual.

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:

Reagent	Content	
R1	–20 °C 2–8 °C 15 °C 20–25 °C	6 month 4 weeks 48 h (on board) 8 h (on board)
R2	–20 °C 2–8 °C 15 °C 20–25 °C	6 month 4 weeks 48 h (on board) 8 h (on board)
CAL	–20 °C 20–25 °C	6 months 8 h (on-board)
C1	–20 °C 20–25 °C	6 months 8 h (on-board)
C2	–20 °C 20–25°C	6 months 8 h (on-board)

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

BLOOD COLLECTION AND SAMPLE PREPARATION

The patient should be at rest for 10 min prior sampling. Collect venous blood carefully in 104 mM sodium citrate (volume ratio 9+1). Gently mix blood and anticoagulant directly aft er 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. Never use a hemolytic plasma sample.
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 in a 37 °C water bath. For more information see NCCLS document H21-A2 [11].

MANUAL PROCEDURE

Reagents should be prepared as described above. Frozen samples should rapidly be thawed at 37 °C in a standardized way ensuring negligible loss of activity of labile coagulation factors and absence of cryoprecipitate.

Preparation of calibrator

Reconstitute in 1.0 ml of deionized water, incubate in closed vials for 15 min at room temperature and swirl gently before use.
Prepare dilutions of calibrator plasma as follows:

Dilutions	Calibrator plasma (µl)	Deionized water (µl)
Dilution 1	0	100
Dilution 2	30	70
Dilution 3	70	30
Dilution 4	100	0

TAFIa activity of undiluted calibrator plasma is lot specific and given in the attached certificate. Calculate TAFIa activity for each dilution.
Either by using a microtiter plate or an automated coagulation analyzer run the test with each calibrator dilution obtained and create a calibration curve by plotting % TAFIa activity values against delta mE per min values obtained in the test.

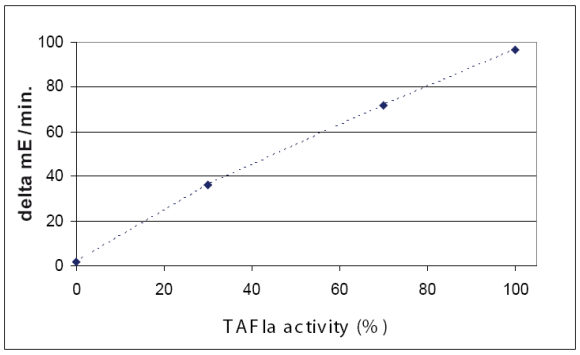
Preparation of Controls
Reconstitute in 1.0 ml of deionized water, incubate in closed vials for 15 min at room temperature and swirl gently before use. After reconstitution controls are ready to use.

Preparation of plasma samples
Dilute **plasma sample 1:2** (one part plasma and one part of 0.9% sodium chloride).
Only dilute plasma samples. **Do not further dilute** dilutions of calibration plasma, and **do not dilute** controls.

Determination and calculation of TAFI activity:
1. Add 10 µL of plasma sample, calibrator dilution, or control to a microwell.
2. Add 100 µL of R1 Activator
3. Incubate the plate for 3 minutes at 37 °C
4. Add 100 µL R2 Start Reagent

Start kinetic measurement at 405 nm. Monitor the rise in extinction over 5 min. Take the linear part of the curve and calculate the slope per minute (ΔE/min). Calculate TAFIa activity by using the calibration curve. Use an appropriate curve fit soft ware or calculate directly using the printed curve.
Consider always the dilution factor of the plasma sample.

Example for a typical calibration curve



AUTOMATED PROCEDURE
Adaptation protocols for automated coagulation analyzers are available. Please contact American Diagnostica.

EXPECTED VALUES
The calibrator plasma is a pooled plasma obtained from normal healthy donors. Calibrator and control plasmas were calibrated against the Secondary Coagulation Standard (SSC/ISTH, Lot. 2). TAFIa activity (%) of undiluted calibrator, controls 1 and 2 is lot specific and given in the attached certificate.
Compare calculated % TAFIa activities of controls 1 and 2 with certified values. If values outside these ranges are obtained the test results are not valid.

QUALITY CONTROLS
Calibrator and control plasma should be used for validation of the assay. Ranges of expected TAFI activities are provided with each batch. If values outside the specified range are obtained, a complete check of reagents should be made and the analysis should be repeated. If the problems persist, a complete instrument check should be considered. In case of further issues please contact technical support for assistance.

SELECTIVITY
The substrate is selective for TAFIa. Use of potato tube carboxypeptidase inhibitor (PTCI) shows a selective inhibition of TAFIa (12, 13), that can be measured by TAFI Activity Kit. Carboxypeptidase N may also cleave the substrate used. Without Thrombin/Thrombomodulin activation of TAFI an activity of 2–4% was found. This negligible activity was probably caused by Carboxypeptidase N activity.

REPRODUCIBILITY
In a series of 36 duplicate determinations on the same day the CV for the microtiter plate assay was below 5%.
In a series of 20 measurements with two lots of reagent on the same day CV for a fully automated coagulation analyzer was below 5% and between two days below 7%.

LIMITATIONS AND INTERFERENCES
Up to now there are no known interferences. Different deficient plasmas (FI, FII, FV, FX, FVIII, Prot. S, Prot. C, ATIII) and factor enriched plasmas (ATIII, TFPI, FVIII, Fibrinogen) were tested. They had no influence on the test outcome. There is also no influence of Lupus anticoagulant antibodies and hemolytic plasma.
The prescribed assay procedure allows the analysis of plasma from anticoagulated patients at heparin levels < 8 U/ml (UFH and LMWH). Please note that the effect of Hirudin anticoagulation is not inhibited by Polybrene.

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