Pefakit® TAFI

Code 800186

For research use only

Intended Use and Application
Pefakit® TAFI is a plasma based chromogenic assay for determination of Thrombin Activatable Fibrinolysis Inhibitor (TAFI) enzyme activity.

Introduction
TAFI (Thrombin Activatable Fibrinolysis Inhibitor) 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 of 309 aa.
- Mode of action of TAFIa: basic carboxypeptidase, cleaves C-terminal lysine and more specifically 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-mercaptop-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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Content</th>
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</table>
| R1      | Activator  
(Pefabloc® FG, DTNB, CaCl₂, Polybrene, Thrombin, Thrombomodulin, Tris, Aprotinin, Pronex®)  
2 vials (lyophilisate, to be reconstituted in 4.0 ml of deionized water) |
| R2      | Start Reagent  
(Substrate, Tris, Mannit)  
2 vials (lyophilisate, to be reconstituted in 4.0 ml of Diluent (R3)) |
| R3      | Diluent  
(10% ethanol, water)  
2 vials ready to use diluent for reconstitution of the Start Reagent (R2) |

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:

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Stability</th>
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| R1      | -20°C: 6 month  
2°C: 4 weeks  
15°C: 48 h (on board)  
20–25°C: 8 h (on board) |
| R2      | -20°C: 6 month  
2°C: 4 weeks  
15°C: 48 h (on board)  
20–25°C: 8 h (on board) |

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

Quality Controls
Calibrator and control plasma delivered in a separate test kit (Pefakit® TAFI Calibrator and Controls, Code 800187) 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.

Preparation and use of Calibrator and Controls
(Pefakit® TAFI Calibrator and Controls, Code 800187)
Use the kit according to box insert instructions. Expected values are given in a certificate attached to the box insert included in that kit.

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 after 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].

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Stability of undiluted samples:
-80 °C: at least 1 year
-20 °C: 2 month
2-8 °C: 12 hours
15-25 °C: 4 hours

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.

Dilute sample plasma 1:2
(one part plasma and one part 0.9% sodium chloride).

Only dilute sample plasmas. Do not further dilute dilutions of calibration plasma, prepared according to box insert instructions of Pefakit® TAFI Calibrator and do not dilute controls.

Determination and calculation of TAFI activity:

<table>
<thead>
<tr>
<th>Plasma</th>
<th>10 µl</th>
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<tbody>
<tr>
<td>R1</td>
<td>100 µl</td>
</tr>
<tr>
<td>Activator</td>
<td></td>
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<tr>
<td>Incubation</td>
<td>3 min, 37°C</td>
</tr>
<tr>
<td>R2</td>
<td>100 µl</td>
</tr>
<tr>
<td>Start Reagent</td>
<td></td>
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</tbody>
</table>

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 TAFI activity by using the calibration curve obtained with the Pefakit® TAFI Calibrator and Controls (Code 800187). Use an appropriate curve fit software or calculate directly using the printed curve.

Consider always the dilution factor of the plasma sample.

Automated Procedure
Adaptation protocols for automated coagulation analyzers are available. Please contact Pentapharm.

Selectivity
The substrate is selective for TAFIa. Use of potato tuber 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 (F1, FII, FV, FVIII, Prot. S, Prot. C, ATIII) and factor enriched plasmas (ATIII, TFP, FVII, 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 Polybriene.

Bibliography

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