INTENDED USE
The TAFI Activity Kit is a plasma based chromogenic assay for the 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 1000 fold over basal rates and is activated to TAFIa by thrombin and probably plasmin [3]. High levels of TAFIa may be an indication for fibrinolysis is most effectively inhibited [3, 4, 5]. High levels of TAFIa may be an indication of thrombotic risk [7, 8, 9, 10].

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 54mercapto-2,4-dinitrobenzoic acid. The extinction measurable at the wavelength of 405 nm 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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<tbody>
<tr>
<td>R1</td>
<td>Activator&lt;br&gt;2 vials, lyophilised&lt;br&gt;(to be reconstituted in 4.0 ml of deionized water)</td>
</tr>
<tr>
<td>R2</td>
<td>Start Reagent&lt;br&gt;2 vials; lyophilised&lt;br&gt;(to be reconstituted in 4.0 ml of Diluent R3)</td>
</tr>
<tr>
<td>R3</td>
<td>Diluent&lt;br&gt;2 vials, ready to use&lt;br&gt;(diluent for reconstitution of the Start Reagent R2)</td>
</tr>
<tr>
<td>CAL</td>
<td>TAFI Calibrator (human plasma)&lt;br&gt;1 vial, lyophilised&lt;br&gt;(to be reconstituted in 1.0 ml of deionized water)</td>
</tr>
<tr>
<td>C1</td>
<td>TAFI Control 1 (human plasma)&lt;br&gt;1 vial, lyophilised&lt;br&gt;(to be reconstituted in 1.0 ml of deionized water)</td>
</tr>
<tr>
<td>C2</td>
<td>TAFI Control 2 (human plasma)&lt;br&gt;1 vial, lyophilised&lt;br&gt;(to be reconstituted in 1.0 ml of deionized water)</td>
</tr>
</tbody>
</table>

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<br>Calibrated pipettes (10–5000 µl)<br>Microtiter plates<br>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>
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<tbody>
<tr>
<td>R1</td>
<td>–20 °C 6 month&lt;br&gt;2–8 °C 4 weeks&lt;br&gt;15 °C 48 h (on board)&lt;br&gt;20–25 °C 8 h (on board)</td>
</tr>
<tr>
<td>R2</td>
<td>–20 °C 6 month&lt;br&gt;2–8 °C 4 weeks&lt;br&gt;15 °C 48 h (on board)&lt;br&gt;20–25 °C 8 h (on board)</td>
</tr>
<tr>
<td>CAL</td>
<td>–20 °C 6 months&lt;br&gt;20–25 °C 8 h (on board)</td>
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<tr>
<td>C2</td>
<td>–20 °C 6 months&lt;br&gt;20–25 °C 8 h (on board)</td>
</tr>
</tbody>
</table>

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 after sampling, avoid foam formation. Centrifuge immediately at no less than 2000 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:

<table>
<thead>
<tr>
<th>Dilutions</th>
<th>Calibrator plasma (µl)</th>
<th>Deionized water (µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution 1</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Dilution 2</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>Dilution 3</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Dilution 4</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>
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 software 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, 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.

BIBLIOGRAPHY
4. The profibrinolytic effect of activated protein C in clots formed from plasma is TAFI-dependent. Bajzar L et al., Blood 1996; 88: 2093-100

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