ab108906

Tissue Factor Human Chromogenic Activity Assay Kit

Instructions for Use

For the quantitative measurement of Human Tissue Factor activity in plasma, serum, urine, tissue and cell culture extracts

This product is for research use only and is not intended for diagnostic use.

Version: 16g Last Updated: 30 March 2020
# Table of Contents

1. Introduction 3
2. Assay Summary 4
3. Kit Contents 5
4. Storage and Handling 5
5. Additional Materials Required 6
6. Preparation of Reagents 6
7. Assay Method 9
8. Data Analysis 10
9. Troubleshooting 12
1. Introduction

The transmembrane protein Tissue factor (TF) is the physiologic trigger of coagulation in normal hemostasis. Tissue factor binds and allosterically activates factor VII (FVII). The TF-FVIIa complex cleaves factor IX and X, leading to thrombin generation. Tissue factor markedly enhances the ability of FVIIa to cleave both macromolecule and small peptidyl substrates. Inducible expression of Tissue factor in a variety of pathological conditions, including gram-negative sepsis and acute coronary syndromes, is associated with life-threatening thrombosis. In sepsis, Tissue factor expression within the vasculature leads to disseminated intravascular coagulation. Tissue factor also plays important roles in vasculogenesis, metastasis, and tumor-associated angiogenesis.

ab108906 Tissue Factor Human Chromogenic Activity Assay kit is developed to determine Human Tissue Factor activity in plasma, serum, urine, tissue and cell culture extracts. The assay measures the ability of lipoprotein TF/FVIIa to activate factor X (FX) to factor Xa. The amidolytic activity of the TF/FVIIa complex is quantitated by the amount of FXa produced using a highly specific FXa substrate releasing a yellow para-nitroaniline (pNA) chromophore. The change in absorbance of the pNA at 405 nm is directly proportional to the TF enzymatic activity.
2. Assay Summary

Prepare all reagents, samples and standards as instructed.

↓

Add 70 μl Assay Mix to each well.

↓

Add 10 μl Tissue Factor standard or samples to each well. Incubate for 30 minutes at 37°C.

↓

Add 20 μl FXa Substrate to each well. Incubate at 37°C. Read the absorbance at 405 nm every 5 minutes for 25 minutes.
3. Kit Contents

- Tissue Factor Microplate: A 96-well polystyrene microplate (12 strips of 8 wells).
- Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- Sample Diluent: (11 ml).
- Assay Diluent: (20 ml).
- Human FVII: (1 vial).
- Human FX: (1 vial).
- FXa Substrate: (2 vials).

4. Storage and Handling

Store Standard, Factor VII protein, Factor X protein and FXa Substrate at -20°C. Store Microplate, Sample Diluent, Assay Diluent at 2 – 8°C. Opened Diluent may be stored for up to 1 month at 2 – 8°C.
5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 μL to 1 mL volumes.
- Distilled or deionized reagent grade water.
- Incubator at 37°C.

6. Preparation of Reagents

Sample Collection:

1. **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and use supernatants. Dilute samples 1:2 into Sample Diluent or within the range of 1x – 5x and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (Heparin can also be used as an anticoagulant, Sodium Citrate is not recommended).

2. **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. Dilute samples 1:2 into Sample Diluent or within the range of 1x – 5x, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
3. **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

4. **Cell Culture Extracts:** It is recommended to use 1 – 2 ml 15 mM octyl-β-D-glucopyranoside to 75 cm² flask cell with 80 % confluent at 37°C for 15 minutes. Collect fresh cell lysates and assay. The samples can be stored at -20°C or below for up to 3 months.

5. **Tissue:** 200 mg tissue can be used with 3-5 ml 50 mM Tris-buffered saline (pH 8.0) with 1% Triton X-100 and centrifuge at 14000 x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:4 into Sample Diluent and assay. Freeze the remaining extract at -20°C.

**Reagent Preparation:**

1. **FVII:** Add 1.2 ml reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.

2. **FX:** Add 1.2 ml reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.

3. **FXa:** Add 1.1 ml reagent grade water. Any remaining solution should be frozen at -20°C and used within 30 days.

4. **Standard Curve:** Standard Curve: Reconstitute the TF Standard with reagent grade water to generate a solution of 500 pM. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard.
solution (500 pM) 1:2 with Sample Diluent to produce 250, 125, 62.5, 31.25, 15.63 and 7.81 pM. Sample Diluent serves as the zero standard (0 pM).

Any remaining TF Standard solution can be stored in single-use aliquots at -20°C for up to 30 days.

<table>
<thead>
<tr>
<th>Standard Point</th>
<th>Dilution</th>
<th>[Tissue Factor] (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1 part Standard (500 pM) + 1 parts Sample Diluent</td>
<td>250.00</td>
</tr>
<tr>
<td>P2</td>
<td>1 part P1 + 1 parts Sample Diluent</td>
<td>125.00</td>
</tr>
<tr>
<td>P3</td>
<td>1 part P2 + 1 parts Sample Diluent</td>
<td>62.5</td>
</tr>
<tr>
<td>P4</td>
<td>1 part P3 + 1 parts Sample Diluent</td>
<td>31.25</td>
</tr>
<tr>
<td>P5</td>
<td>1 part P4 + 1 parts Sample Diluent</td>
<td>15.63</td>
</tr>
<tr>
<td>P6</td>
<td>Sample Diluent</td>
<td>7.81</td>
</tr>
<tr>
<td>P7</td>
<td>Sample Diluent</td>
<td>0.00</td>
</tr>
</tbody>
</table>
7. Assay Method

1. Prepare all reagents, working standards and samples as instructed.

2. Freshly prepare the desired volume of Assay Mix by combining the following reagents as described below. The values represent the volumes for one reaction:

   - Assay Diluent 50 μL
   - FVII 10 μL
   - FX 10 μL

3. Add 70 μL of the Assay Mix to each well.

4. Add 10 μL of Tissue Factor Standard or sample to each well. Mix gently. Incubate at 37°C for 30 minutes in a humid incubator to avoid drying out the wells.

5. Add 20 μL of FXa Substrate to each well and mix gently. Cover wells with a sealing tape and incubate at 37°C and read absorbances on a microplate reader at a wavelength of 405 nm every 5 minutes for 25 minutes.
8. Data Analysis

Calculate the mean value of the triplicate readings for each standard and sample. Determine the optimal time frame in which the various standard concentrations have steady OD increase, and use this optimal time frame data for standard curve plotting and data analysis. Determine ΔOD/min, ie total OD increase of sample over total time frame and divide by number of minutes/time frame. Plot the standard curve (x-axis: TF concentration; y-axis: OD405) using standard readings calculated above. Determine the best-fit line by regression analysis of the 4 parameter curve. Interpolate the sample concentration using the sample readings from the linear part of the standard curve.

A. Typical Data

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.

This assay recognizes both natural and recombinant Human Tissue Factor.
B. Sensitivity

The minimum detectable dose of Tissue Factor is typically <7.5 p
## 9. Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor standard curve</td>
<td>Improper standard dilution</td>
<td>Confirm dilutions made correctly</td>
</tr>
<tr>
<td></td>
<td>Standard improperly reconstituted (if applicable)</td>
<td>Briefly spin vial before opening; thoroughly resuspend powder (if applicable)</td>
</tr>
<tr>
<td></td>
<td>Standard degraded</td>
<td>Store sample as recommended</td>
</tr>
<tr>
<td></td>
<td>Curve doesn't fit scale</td>
<td>Try plotting using different scale</td>
</tr>
<tr>
<td>Low signal</td>
<td>Incubation time too short</td>
<td>Try overnight incubation at 4°C</td>
</tr>
<tr>
<td></td>
<td>Target present below detection limits of assay</td>
<td>Decrease dilution factor; concentrate samples</td>
</tr>
<tr>
<td></td>
<td>Precipitate can form in wells upon substrate addition when concentration of target is too high</td>
<td>Increase dilution factor of sample</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
<tr>
<td></td>
<td>Sample prepared incorrectly</td>
<td>Ensure proper sample preparation/dilution</td>
</tr>
<tr>
<td>Problem</td>
<td>Cause</td>
<td>Solution</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------------</td>
<td>--------------------------------------------------------------------------</td>
</tr>
<tr>
<td>High background</td>
<td>Wells are insufficiently washed</td>
<td>Wash wells as per protocol recommendations</td>
</tr>
<tr>
<td></td>
<td>Contaminated wash buffer</td>
<td>Make fresh wash buffer</td>
</tr>
<tr>
<td></td>
<td>Waiting too long to read plate after adding STOP solution</td>
<td>Read plate immediately after adding STOP solution</td>
</tr>
<tr>
<td>Large CV</td>
<td>Bubbles in wells</td>
<td>Ensure no bubbles present prior to reading plate</td>
</tr>
<tr>
<td></td>
<td>All wells not washed equally/thoroughly</td>
<td>Check that all ports of plate washer are unobstructed/wash wells as recommended</td>
</tr>
<tr>
<td></td>
<td>Incomplete reagent mixing</td>
<td>Ensure all reagents/master mixes are mixed thoroughly</td>
</tr>
<tr>
<td></td>
<td>Inconsistent pipetting</td>
<td>Use calibrated pipettes and ensure accurate pipetting</td>
</tr>
<tr>
<td></td>
<td>Inconsistent sample preparation or storage</td>
<td>Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)</td>
</tr>
<tr>
<td>Low sensitivity</td>
<td>Improper storage of ELISA kit</td>
<td>Store all reagents as recommended. Please note all reagents may not have identical storage requirements.</td>
</tr>
<tr>
<td></td>
<td>Using incompatible sample type (e.g. Serum vs. cell extract)</td>
<td>Detection may be reduced or absent in untested sample types</td>
</tr>
</tbody>
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For further technical questions please do not hesitate to contact us by email (technical@abcam.com) or phone (select
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