AssaySense
Human Tissue Factor Chromogenic Activity Kit

Assaypro LLC
3400 Harry S Truman Blvd
St. Charles, MO 63301
T (636) 447-9175
F (636) 395-7419
www.assaypro.com

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@assaypro.com.

Thank you for choosing Assaypro.
Assay Summary

**Step 1.** Add 70 µl of Assay Mix and add 10 µl of Standard or Sample to each well. Incubate at 37°C for 30 minutes.

**Step 2.** Add 20 µl of Factor Xa Substrate to each well.

**Step 3.** Read the absorbance at 405 nm at zero minutes. Incubate at 37°C. Read at 405 nm every 5 minutes for 25 minutes.

Symbol Key

📖 Consult instructions for use
Introduction

The transmembrane protein tissue factor (TF) is the physiologic trigger of coagulation in normal hemostasis. TF binds and allosterically activates factor VII (FVII). The TF-FVIIa complex cleaves factor IX and X, leading to thrombin generation (1). TF markedly enhances the ability of FVIIa to cleave both macromolecule and small peptidyl substrates (2, 3). Inducible expression of TF in a variety of pathological conditions, including gram-negative sepsis and acute coronary syndromes, is associated with thrombosis (4, 5). TF also plays important roles in vasculogenesis, metastasis, and angiogenesis (6-8).

Principle of the Assay

The AssaySense Human Tissue Factor Chromogenic Activity Kit is developed to determine human TF chromogenic activity in plasma, serum, urine, tissue, and cell culture samples. 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.

Caution and Warning

- This product is for Research Use Only and is not intended for use in diagnostic procedures.
- Prepare all reagents as instructed, prior to running the assay.
- Prepare all samples prior to running the assay. The dilution factors for the samples are suggested in this insert. However, the user should determine the optimal dilution factor.
- The kit should not be used beyond the expiration date.

Reagents

The activity kit contains sufficient reagents to perform 100 tests using the microplate method.
- **Microplate**: One 96-well polystyrene microplate (12 strips of 8 wells)
- **Sealing Tapes**: Each kit contains 3 precut, pressure sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Sample Diluent (1x)**: 11 ml
- **Assay Diluent (1x)**: 20 ml
- **rhTF Standard (Lipoprotein)**: Recombinant human TF lipoprotein (1500 pM, lyophilized, 1 vial)
- **Human Factor VII**: Lyophilized, 1 vial
- **Human Factor X**: Lyophilized, 1 vial
- **FXa Substrate**: Lyophilized, 2 vials

**Storage Condition**

- Upon arrival, immediately store components of the kit at recommended temperatures up to the expiration date.
- Store Standard, Factor VII, Factor X, and FXa Substrate at -20°C.
- Store Microplate, Sample Diluent, and Assay Diluent at 2-8°C.
- Unused microplate wells may be returned to the pouch and resealed.
- Opened diluent may be stored for up to 30 days at 2-8°C.

**Other Supplies Required**

- Microplate reader capable of measuring absorbance at 405 nm.
- Pipettes (1-20 μl, 20-200 μl, 200-1000 μl, and multiple channel).
- Deionized or distilled reagent grade water.
- Incubator (37°C).

**Sample Collection, Preparation, and Storage**

- **Plasma**: Collect plasma using EDTA as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. If necessary, dilute samples using Sample Diluent within the range of 1x – 5x. 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.

- **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. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. If necessary, dilute samples using Sample Diluent within the range of 1x – 5x. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
• **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. The sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

• **Cell Culture Lysates:** The cultured cells are lysed and solubilized with 15 mM octyl-β-D-glucopyranoside at 37°C for 15 minutes. Collect fresh cell lysates. Samples can be stored at -20°C or below.

• **Tissue:** Extract tissue samples using 50 mM Tris-buffered saline (pH 8.0) with 1% Triton X-100 and centrifuge at 14000 x g for 20 minutes. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 4-fold into Sample Diluent. The undiluted extract can be stored at -20°C or below.

**Reagent Preparation**

• **rhTF Standard:** Reconstitute the rhTF Standard (1500 pM) with 3 ml of reagent grade water to generate a 500 pM standard stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting from the standard stock solution (500 pM) 2-fold with equal volume of Sample Diluent to produce 250, 125, 62.5, 31.25, 15.625, and 7.813 pM solutions. Sample Diluent serves as the zero standard (0 pM). Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

<table>
<thead>
<tr>
<th>Standard Point</th>
<th>Dilution</th>
<th>[rhTF] (pM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1 part Standard (500 pM) + 1 part Sample Diluent</td>
<td>250</td>
</tr>
<tr>
<td>P2</td>
<td>1 part P1 + 1 part Sample Diluent</td>
<td>125</td>
</tr>
<tr>
<td>P3</td>
<td>1 part P2 + 1 part Sample Diluent</td>
<td>62.5</td>
</tr>
<tr>
<td>P4</td>
<td>1 part P3 + 1 part Sample Diluent</td>
<td>31.25</td>
</tr>
<tr>
<td>P5</td>
<td>1 part P4 + 1 part Sample Diluent</td>
<td>15.625</td>
</tr>
<tr>
<td>P6</td>
<td>1 part P5 + 1 part Sample Diluent</td>
<td>7.813</td>
</tr>
<tr>
<td>P7</td>
<td>Sample Diluent</td>
<td>0.0</td>
</tr>
</tbody>
</table>

• **Human Factor VII:** Add 1.2 ml reagent grade water to produce a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation. Any remaining stock solution should be stored at -20°C and used within 30 days.

• **Human Factor X:** Add 1.2 ml reagent grade water to produce a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation. Any remaining stock solution should be stored at -20°C and used within 30 days.
- **FXa Substrate:** Add 1.1 ml reagent grade water to produce a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation. Any remaining stock solution should be stored at -20°C and used within 30 days.

**Assay Procedure**

- Prepare all reagents, standard solution, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C in a humid incubator.
- Remove excess microplate strips from the plate frame.
- Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n) plus one well.

<table>
<thead>
<tr>
<th>Assay Mix</th>
<th>Reagent</th>
<th>n = 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Assay Diluent</td>
<td>50 µl</td>
</tr>
<tr>
<td></td>
<td>Human FVII</td>
<td>10 µl</td>
</tr>
<tr>
<td></td>
<td>Human FX</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

- Add 70 µl of the Assay Mix to each well. Gently tap plate to thoroughly coat the wells.
- Add 10 µl of rhTF Standard or sample to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape, and incubate at 37°C for 30 minutes in a humid incubator to avoid evaporation.
- Add 20 µl of FXa Substrate to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405 nm at zero minutes for background OD.
- Cover wells with a sealing tape and incubate at 37°C in a humid incubator to avoid evaporation. Read the absorbance at 405 nm every 5 minutes for 25 minutes.

<table>
<thead>
<tr>
<th>Add 70 µl Assay Mix</th>
<th>Add 10 µl rhTF Standard or Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incubate at 37°C for 30 minutes</td>
<td>Add 20 µl FXa Substrate</td>
</tr>
<tr>
<td>Read absorbance at 405 nm at zero minutes for background OD. Incubate at 37°C. Read absorbance at 405 nm every 5 minutes for 25 minutes.</td>
<td></td>
</tr>
</tbody>
</table>
**Data Analysis**

- Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
- To generate a standard curve from the optimal reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance (OD) on the y-axis. The best-fit line can be determined by regression analysis of the 4-parameter curve.
- Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

**Standard Curve**

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

![Tissue Factor Chromogenic Activity Standard Curve](image)

**Performance Characteristics**

- The minimum detectable dose of human TF is approximately 7.5 pM.
- This assay recognizes both natural and recombinant human TF.
## Troubleshooting

<table>
<thead>
<tr>
<th>Issue</th>
<th>Causes</th>
<th>Course of Action</th>
</tr>
</thead>
</table>
| **Low Precision** | Use of expired components | - Check the expiration date listed before use.  
- Do not interchange components from different lots. |
|  | Splashing of reagents while loading wells | - Pipette properly in a controlled and careful manner. |
|  | Inconsistent volumes loaded into wells | - Pipette properly in a controlled and careful manner.  
- Check pipette calibration.  
- Check pipette for proper performance. |
|  | Insufficient mixing of reagent dilutions | - Thoroughly agitate the lyophilized components after reconstitution.  
- Thoroughly mix dilutions. |
| **Unexpectedly Low or High Signal Intensity** | Microplate was left unattended between steps | - Each step of the procedure should be performed uninterrupted. |
|  | Omission of step | - Consult the provided procedure for complete list of steps. |
|  | Steps performed in incorrect order | - Consult the provided procedure for the correct order. |
|  | Insufficient amount of reagents added to wells | - Check pipette calibration.  
- Check pipette for proper performance. |
|  | Improper reagent preparation | - Consult reagent preparation section for the correct dilutions of all reagents. |
|  | Insufficient or prolonged incubation periods | - Consult the provided procedure for correct incubation time. |
| **Deficient Standard Curve Fit** | Non-optimal sample dilution | - User should determine the optimal dilution factor for samples. |
|  | Contamination of reagents | - A new tip must be used for each addition of different samples or reagents during the assay procedure. |
|  | Contents of wells evaporate | - Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature. |
|  | Improper pipetting | - Pipette properly in a controlled and careful manner.  
- Check pipette calibration.  
- Check pipette for proper performance. |
|  | Insufficient mixing of reagent dilutions | - Thoroughly agitate the lyophilized components after reconstitution.  
- Thoroughly mix dilutions. |
References

(4) Fuster, V. et al. (1996) Haemostasis 26:269