AssaySense

Human Tissue Factor Chromogenic Activity Kit

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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 per well.
Add 10 µl of Standard or Sample per well.
Cover and incubate for 30 minutes at 37°C.

Step 2. Add 20 µl of FXa Substrate per well.
Read the absorbance at 405 nm for a zero minute background reading.
Cover and incubate at 37°C.

Step 3. Read every 5 minutes for 35 minutes at 405 nm.
Cover and incubate at 37°C after each reading.

Symbol Key

Consult instructions for use

Assay Template

	A	B	C	D	E	F	G	H
1								
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AssaySense Human Tissue Factor (TF) Chromogenic Activity Kit (Two Step, Lipoprotein)

Catalog No. CT1002b

Sample insert for reference use only

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 (FIX) and factor X (FX), 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 life-threatening thrombosis (4-5). In sepsis, TF expression within the vasculature leads to disseminated intravascular coagulation (6). TF also plays important roles in vasculogenesis, metastasis, and tumor-associated angiogenesis (7-9).

Principle of the Assay

The AssaySense Human Tissue Factor Chromogenic Activity Kit is developed to determine TF chromogenic activity in **human plasma, serum, urine, cell lysate, and tissue samples**. This kit is also validated for use with **canine, bovine, equine, monkey, mouse, rat, swine, and rabbit samples.** The assay measures the ability of lipoprotein TF/FVIIa to activate FX to FXa. 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 assay kit contains sufficient reagents to perform 96 tests using the microplate method.

- **Microplate:** A 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.
- **Human Tissue Factor Standard (Lipoprotein):** Recombinant human TF lipoprotein (0.75 pmol, lyophilized).
- **Human Factor VII:** Lyophilized.
- **Human Factor X:** Lyophilized.
- **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. A human plasma sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. 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. A human serum sample is suggested for use at 1x; however, user

should determine optimal dilution factor depending on application needs. 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. A human urine sample is suggested for use at 1x or within the range of 2x – 5x into Sample Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Lysate:** The cultured cells are lysed and solubilized with 50 mM Tris-buffered saline (pH 8.0) containing 0.5% Triton X-100 or 0.1% Tween 20 on ice for 30 minutes. Centrifuge samples at 14000 rpm for 10 minutes. Collect fresh cell lysate. If necessary, dilute samples into Sample Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples with 50 mM Tris-buffered saline (pH 8.0) containing 0.5% Triton X-100 or 0.1% Tween 20 on ice for 30 minutes. Centrifuge samples at 14000 rpm for 20 minutes. Collect supernatant. If necessary, dilute samples into Sample Diluent; user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Applicable samples may also include biofluids, cell culture, and tissue homogenates. If necessary, user should determine optimal dilution factor depending on application needs.

Refer to Dilution Guidelines for further instruction.

Guidelines for Dilutions of 100-fold or Greater
(*for reference only; please follow the insert for specific dilution suggested)*

100x	**10000x**
A) 4 µl sample : 396 µl buffer (100x) = 100-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) = 10000-fold dilution *Assuming the needed volume is less than or equal to 400 µl.*
1000x	**100000x**
A) 4 µl sample : 396 µl buffer (100x) B) 24 µl of A : 216 µl buffer (10x) = 1000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*	A) 4 µl sample : 396 µl buffer (100x) B) 4 µl of A : 396 µl buffer (100x) C) 24 µl of B : 216 µl buffer (10x) = 100000-fold dilution *Assuming the needed volume is less than or equal to 240 µl.*

Reagent Preparation

- Freshly dilute all reagents and bring all reagents to room temperature before use.
- **Human Tissue Factor Standard:** Reconstitute the Human Tissue Factor Standard (0.75 pmol) with 1.5 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). Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 5 days**.

Standard Point	Dilution	[TF] (pM)
P1	1 part Standard (500 pM) + 1 part Sample Diluent	250
P2	1 part P1 + 1 part Sample Diluent	125
P3	1 part P2 + 1 part Sample Diluent	62.5
P4	1 part P3 + 1 part Sample Diluent	31.25
P5	1 part P4 + 1 part Sample Diluent	15.625
P6	1 part P5 + 1 part Sample Diluent	7.813
P7	Sample Diluent	0.0

- **Human Factor VII:** Add 1.4 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 5 days**.
- **Human Factor X:** Add 1.4 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
- **FXa Substrate:** Add 1.1 ml of reagent grade water to generate a 1x stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and **used within 10 days**.

Assay Procedure

- Prepare all reagents, standard solutions, and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C in a humid incubator to avoid evaporation.
- 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 number of wells in the assay (n) plus one well.

Assay Mix Reagent	n = 1 well
Assay Diluent	50 µl
Human Factor VII	10 µl
Human Factor X	10 µl

- Add 70 µl of Assay Mix to each well. Gently tap plate to thoroughly coat the wells. Add 10 µl of Human Tissue Factor 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 for 30 minutes at 37°C in a humid incubator.
- 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 for a zero minute background reading. Cover wells with a sealing tape and incubate at 37°C in a humid incubator.
- Read the absorbance at 405 nm every 5 minutes for 35 minutes. Cover wells with a sealing tape and incubate at 37°C after each reading.

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) or change in absorbance per minute (ΔA/min) on the y-axis after subtracting the background. 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.

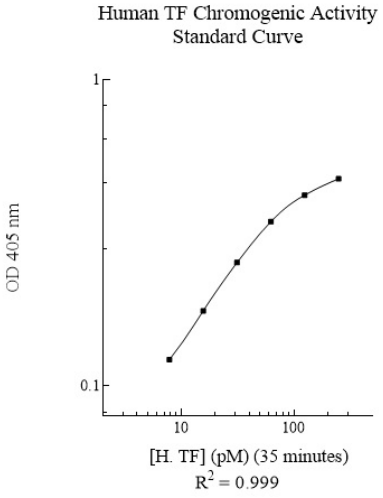
Typical Data

- The typical data is provided for reference only. Individual laboratory means may vary from the values listed. Variations between laboratories may be caused by technique differences.

Standard Point	pM	Average OD
P1	250	0.475
P2	125	0.420
P3	62.5	0.344
P4	31.25	0.253
P5	15.625	0.176
P6	7.813	0.122
P7	0.0	0.055

Standard Curve

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



Performance Characteristics

- This assay recognizes both natural and recombinant human TF.
- The minimum detectable dose of human TF at 35 minutes is approximately 3.5 pM.

Troubleshooting

Issue	Causes	Course of Action
Low Precision	Use of improper 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

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