

AssaySense Human Tissue Factor (TF) Chromogenic Activity Assay Kit (Two Steps, Lipoprotein)

Catalog Number CT1002b

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 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, 8, 9).

Principle of Assay

The AssaySense Human TF Chromogenic Activity Assay Kit is developed to determine human TF chromogenic activity in plasma, tissue, and cell culture supernatants. 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 kit is for research use only.
- The kit should not be used beyond the expiration date.
- All human source materials have been tested and found to be negative to HbsAg, HIV-1 and HCV by FDA approved methods.

Reagents

The activity assay kit contains sufficient reagents to perform 100 tests using microplate method.

- **TF Microplate:** One 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:** 30 ml
- **Assay Diluent :** 20 ml
- **rhTF Standard (lipoprotein):** 1 vial recombinant human TF lipoprotein
- **Human FVII:** 1 vial
- **Human FX:** 1 vial
- **FXa Substrate:** 2 vials

Storage Condition

- Store unopened kit at 2-8⁰C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8⁰C. Store reconstituted standard and reagents at -20⁰C or below.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 μ l, 20-200 μ l, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37⁰C)

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000 x g for 10 minutes and assay. The undiluted samples can be stored at -20⁰C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
- **Cell Culture Supernatants:** The cultured cells are lysed and solubilized with 15 mM octyl- β -D-glucopyranoside at 37⁰C for 15 minutes. Collect fresh cell lysates and assay. The samples can be stored at -20⁰C or below for up to 3 months
- **Tissue:** Extract tissue samples using 50 mM Tris-buffered saline (pH 8.0) with 1% Triton X-100 and centrifuge at 14,000 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 < -20⁰C.

Reagent Preparation

- **Standard Curve:** Reconstitute the TF Standard with 4 ml of reagent grade water to generate a solution of 2 nM. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare triplicate standard points by serially diluting the standard solution (2 nM) 1:4 with Sample Diluent to produce 500, 125, 31.25, 7.81, 1.95 and 0.49 pM. Sample Diluent serves as the zero standard (0 pM).

Standard Point	Dilution	[TF] (pM)
P1	1 part Standard (1nM) + 3 part Sample Diluent	500.00
P2	1 part P1 + 3 part Sample Diluent	125.00
P3	1 part P2 + 3 part Sample Diluent	31.25
P4	1 part P3 + 3 part Sample Diluent	7.81
P5	1 part P4 + 3 part Sample Diluent	1.95
P6	1 part P5 + 3 part Sample Diluent	0.49
P7	Sample Diluent	0.00

- **FVII:** Add 1.2 ml reagent grade water.
- **FX:** Add 1.2 ml reagent grade water.
- **FXa Substrate:** Add 1.1 ml reagent grade water.

Assay Procedure

- Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n).

Reagents	n=1
Assay Diluent	50 μ l
FVII	10 μ l
FX	10 μ l

- Add 70 μ l of the above Assay Mix to each well of the 96-well plate.
- Add 10 μ l of TF Standards or samples per well of the 96-well plate. Mix gently.
- Incubate at 37^oC for 30 minutes.
- Add 20 μ l of FXa Substrate to each well and mix gently. Incubate at 37^oC and read the absorbances at 405 nm every 2 minute for 6 minutes.

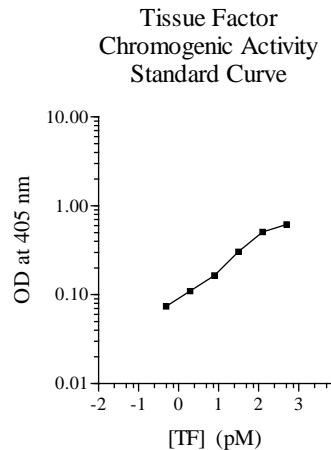
Assay Mix	70 μ l
TF or Samples	10 μ l
<i>37^oC, 30 minutes</i>	
FXa Substrate	20 μ l
<i>37^oC, Read the absorbances at 405 nm every 2 minutes for 6 minutes</i>	

Data Analysis

- Calculate the mean value of the triplicate for each standard and sample.
- To generate 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 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.

Standard Curve

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



Performance Characteristics

- The minimum detectable dose of TF is typically < 0.4 pM.
- This assay recognizes both natural and recombinant human TF.

References

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Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium