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

Catalog Number CT1003b

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 AngioSense 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 TF/FVIIa to activate factor X (FX) to factor Xa in the presence of phospholipids coated to a microplate. 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) coated with phospholipids.
- **Assay Diluent (5x):** 10 ml
- **rhTF Standard (apoprotein):** 1 vial recombinant human TF apoprotein
- **Human FVII:** 1 vial
- **Human FX:** 1 vial
- **FXa Substrate:** 1 vial
- **Substrate Diluent:** 5 ml

Storage Condition

- Store unopened kit at 2-8^{0}C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8^{0}C. Store reconstituted standard and reagents at -20^{0}C or below.
- Opened unused strip wells may return to the foil pouch with the desiccant pack, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Other Supplies Required

- Microplate reader capable of measuring absorbance at 405 nm
- Pipettes (1-20 µl, 20-200 µl, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37^{0}C)

Sample Collection, Preparation and Storage

- **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3,000x g for 10 minutes and assay. Dilute samples 1:2 with Assay Diluent. Store samples at -20^{0}C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

- **Cell Culture Supernatants:** Collect cell culture media and centrifuge at 3,000 x g for 10 minutes at 4^{0}C to remove debris. Samples can be store at < -20^{0}C. Avoid repeated freeze-thaw cycles.
- **Tissue:** Extract tissue samples using Tris-buffered saline (pH 8.0) with 1% Triton X-100 and centrifuge at 14,000x g for 20 min. Collect the supernatant and measure the protein concentration. Dilute the tissue extract 1:10 into Assay Diluent and assay. Freeze the remaining extract at < -20^{0}C.

Reagent Preparation

- **Standard Curve:** Reconstitute the TF Standard with 1.5 ml of Assay Diluent to generate a solution of 0.4 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 (0.4 nM) twofold with equal volume of Assay Diluent to produce 0.2, 0.1, 0.05, 0.025, 0.0125, and 0.00625 nM. Assay Diluent serves as the zero standard (0 nM).

Standard Point	Dilution	[TF] (nM)
P1	1 part Standard (0.4 nM) + 1 part Assay Diluent	0.2000
P2	1 part P1 + 1 part Assay Diluent	0.1000
P3	1 part P2 + 1 part Assay Diluent	0.0500
P4	1 part P3 + 1 part Assay Diluent	0.0250
P5	1 part P4 + 1 part Assay Diluent	0.0125
P6	1 part P5 + 1 part Assay Diluent	0.0063
P7	Assay Diluent	0.0000

- **Assay Diluent (5x)**: Dilute the Assay Diluent 1:5 with reagent grade water.
- **FVII:** Add 1.2 ml Assay Diluent.
- **FX:** Add 1.2 ml Assay Diluent.
- **FXa Substrate**: Add 2.2 ml Substrate Diluent.

Assay Procedure

- Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely and store in a vacuum desiccator to minimize exposure to water vapor.
- Add 20 µl of Assay Dilutent to each well.
- Add 10 µl of FVII and 10 µl of TF Standards or diluted testing samples per well of the 96-well plate. Mix gently.
- Incubate at 37^0C for 30 minutes.
- Add 10 µl FX and 20 µl of FVIIa Substrate to each well and mix gently.
- Incubate at 37 ^{0}C and read the absorbances at 405 nm every 2 minutes.

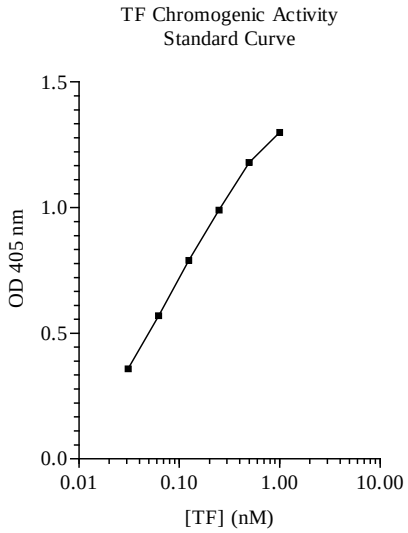
Assay Diluent	20 µl
FVII	10 µl
TF or Samples	10 µl
37^0C, 30 minutes	
FX	10 µl
FXa Substrate	20 µl
37^0C, read the absorbances at 405 nm every 2 minutes	

Data Analysis

- Calculate the mean value of the triplicate for each standard and sample.
- To generate a Standard Curve from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis of the linear portion of the 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.



Performance Characteristics

- The minimum detectable dose of TF is typically < 5 pM.
- This assay recognizes both natural and recombinant human TF. No significant cross-reactivity or interference was observed.

References

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