



# **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.
- **Sample Diluent:** 30 ml
- **Assay Diluent :** 5 ml
- **rhTF Standard (apoprotein):** 1 vial recombinant human TF apoprotein
- **Human FVII:** 1 vial
- **Human FX:** 1 vial
- **FXa Substrate:** 2 vials

## Storage Condition

- Store unopened kit at 2-8<sup>0</sup>C up to expiration date.
- Opened reagents may be stored for up to 1 month at 2-8<sup>0</sup>C. Store reconstituted standard and reagents at -20<sup>0</sup>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  $\mu$ l, 20-200  $\mu$ l, and multiple channel)
- Deionized or distilled reagent grade water
- Incubator (37<sup>0</sup>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 3,000 x g for 10 minutes. Dilute samples 1:4 with Sample Diluent and assay. The undiluted samples can be stored at -20<sup>0</sup>C 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- $\beta$ -D-glucopyranoside at 37<sup>0</sup>C for 15 minutes. Collect fresh cell lysates and assay. The samples can be stored at -20<sup>0</sup>C 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 < -20<sup>0</sup>C.

## Reagent Preparation

- **Standard Curve:** Reconstitute the TF Standard with 0.6 ml of Sample Diluent to generate a solution of 1 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 (1 nM) twofold with equal volume of Sample Diluent to produce 500, 250, 125, 62.5 and 31.25, 15.63 and 7.81 pM. Sample Diluent serves as the zero standard (0 pM).

Standard Point	Dilution	[TF] (pM)
P1	1 part Standard (1nM) + 1 part Sample Diluent	500.00
P2	1 part P1 + 1 part Sample Diluent	250.00
P3	1 part P2 + 1 part Sample Diluent	125.00
P4	1 part P3 + 1 part Sample Diluent	62.50
P5	1 part P4 + 1 part Sample Diluent	31.25
P6	1 part P5 + 1 part Sample Diluent	15.63
P7	1 part P6 + 1 part Sample Diluent	7.81
P8	Sample Diluent	0.00

- **FVII:** Add 0.6 ml reagent grade water
- **FX:** Add 1.2 ml reagent grade water.
- **FXa Substrate:** Add 1.1 ml reagent grade water.

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

Reagents	<u>n=1</u>
FVII	5 $\mu$ l
FX	10 $\mu$ l
Assay Diluent	15 $\mu$ l

- Add 30  $\mu$ l of the above Assay Mix to each well of the 96-well plate.
- Add 10  $\mu$ l of TF Standards or samples per well of the 96-well plate. Mix gently.
- Incubate at 37<sup>0</sup>C for 5 minutes.
- Add 20  $\mu$ l of FXa Substrate to each well and mix gently. Incubate at 37<sup>0</sup>C for 5 minutes. Read the absorbances at 405 nm.

Assay Mix	30 $\mu$ l
TF or Samples	10 $\mu$ l
<i>37<sup>0</sup>C, 5 minutes</i>	
FXa Substrate	20 $\mu$ l
<i>37<sup>0</sup>C, 5 minutes</i>	
Read the absorbances at 405 nm	

### Data Analysis

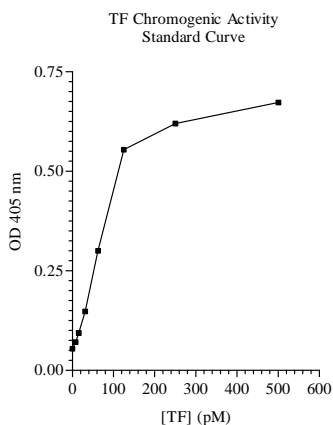
- Calculate the mean value of the triplicate for each standard and sample.
- To generate a Standard Curve from the initial reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in

absorbance per minute ( $\Delta A/\text{min}$ ) 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.

## References

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