AssaySense Human Tissue Factor (TF) Chromogenic Activity Assay Kit (One Step, Apoprotein)

Catalog Number CT1003a

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 lysate. The assay measures the activation of zymogen FVII to FVIIa by TF in the presence of phospholipids coated to a microplate. The amidolytic activity of the TF/FVIIa complex is quantitated using a highly specific FVIIa 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:** A 96 well polystyrene microplate (12 strips of 8 wells) coated with phospholipids.
- **Sealing Tapes:** Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- **Assay Diluent (5x):** 10 ml
- **rhTF Standard (apoprotein):** 1 vial recombinant human TF apoprotein
- **Human FVII:** 1 vial
- **FVIIa Substrate:** 2 vials

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 3,000 x g for 10 minutes and assay. Dilute samples 1:4 with Assay Diluent. Store samples at -20^{0}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-glycopyranoside at 37^{0}C for 15 minutes. Collect cell lysates, dilute with Assay Diluent and assay.
- **Tissue:** Extract tissue samples using 50 mM 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 1:4 into Assay Diluent and assay. Freeze the remaining extract at < -20^{0}C.

Reagent Preparation

- **Standard Curve:** Reconstitute the TF Standard with 1 ml of Assay 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 Assay Diluent to produce 500, 250, 125, 62.5, 31.25 and 15.63 for high level and 7.82, 3.91, 1.95, 0.98 and 0.49 pM for low level of TF detection. Assay Diluent serves as the zero standard (0 pM).

Standard curve for high level of TF activity samples:

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

Standard curve for low level of TF activity samples:

Standard Point	Dilution	[TF] (pM)
P1	1 part Standard (15.63 pM)	15.63
P2	1 part P1 + 1 part Assay Diluent	7.81
P3	1 part P2 + 1 part Assay Diluent	3.91
P4	1 part P3 + 1 part Assay Diluent	1.95
P5	1 part P4 + 1 part Assay Diluent	0.98
P6	1 part P5 + 1 part Assay Diluent	0.49
P7	1 part Assay Diluent	0.00

- **Assay Diluent (5x)**: Dilute the Assay Diluent 1:5 with reagent grade water.
- **rhTF:** Add 0.6 ml Assay Diluent.
- **FVII:** Add 1.2 ml Assay Diluent.
- **FVIIa 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.
- Add 50 µl of Assay Dilutent to each well of the 96-well plate.
- Add 10 µl of FVII and 20 µl of TF Standards or diluted samples per well. Mix gently.
- Incubate at 37^0C for 30 minutes.
- Add 20 µl of FVIIa Substrate to each well and mix gently. Read the absorbance at 405 nm at zero minutes for background O.D. Incubate the samples at 37^0C and monitor the absorbance at 405 nm periodically as below.

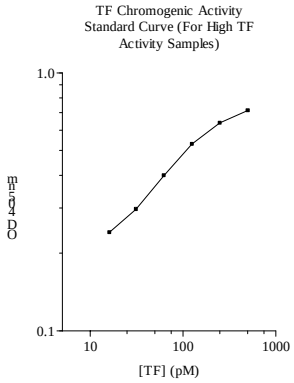
Assay Diluent	50 µl
FVII	10 µl
TF or Samples	20 µl
37^0C, 30 minutes	
FVIIa Substrate	20 µl
High TF activity Samples: Read the absorbance at 405 nm every 5 minutes at *37^0C* for 40 minutes.	
Low TF activity Samples: Read the absorbance at 405 nm every 20 minutes at *37^0C* from 40 minutes to 2 hours.	

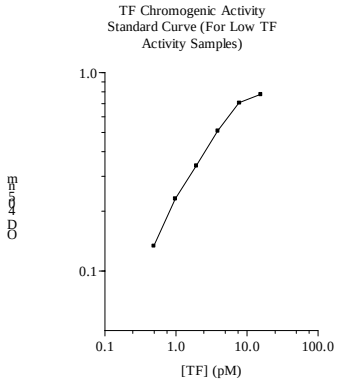
Data Analysis

- 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 after subtracting the background. 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 < 0.5 pM.
- This assay recognizes both natural and recombinant human TF.

References

1. Ruf, W. and Edgington, T.S. (1994) *FASEB J*. 8:385
2. Neuenschwander, P.F. *et al*. (1993) *Thromb. Haemost.*70:970
3. Morrissey, J.H. (1995) *Thromb. Haemost.* 74:185
4. Fuster, V. *et al.* (1996) *Haemostasis* 26:269
5. Leatham, E. *et al*. (1995) *Br. Heart. J.* 73:10
6. Drake, TA. *et al*. (1993) *Am. J. Pathol*. 142:1
7. Carmeliet, P. *et al.* (1996) *Nature* 383:73
8. Ruf, W. and Mueller, B.M. (1996) *Curr. Opin. Hematol*. 3:379
9. Zhang, Y. *et al.* (1994) *J. Clin. Invest*. 94:1320

Revision 4.0