TF (Human) Chromogenic Activity Assay Kit

Catalog Number KA0975

96 assays

Version: 03

Intended for research use only

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Introduction

Background

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 (7-9).

Principle of the Assay

The TF (Human) Chromogenic Activity Assay Kit is developed to determine human TF chromogenic activity in plasma, serum, urine, tissue, and cell culture samples. 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.

General Information

Materials Supplied

List of component

Component	Amount
Microplate: A 96-well polystyrene microplate.	96(8x12) wells
Sealing Tapes: Pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.	3 slices
Sample Diluent	30 mL
Assay Diluent	20 mL
rhTF Standard (Lipoprotein): Recombinant human TF lipoprotein.	1 vial
Human FVII	1 vial
Human FX	1 vial
Fxa Substrate	2 vials

Storage Instruction

- ✓ Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date.
- ✓ Store Standard, Factor VII protein , Factor X protein, 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 foil pouch and resealed. May be stored for up to 30 days in a vacuum desiccator.
- ✓ Opened diluent may be stored for up to 30 days at 2-8°C.

Materials Required but Not Supplied

- ✓ 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).

Precautions for Use

- ✓ 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 protocol. However, the user should determine the optimal dilution factor.
- ✓ 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.

Assay Protocol

Reagent Preparation

✓ Standard Curve

Reconstitute the rhTF Standard with 1.2 mL of reagent grade water to generate solution of 1000 pM. standard stock solution. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (1000 pM) 1:2 with Sample Diluent to produce 500, 250, 125, 62.5, 31.25, and 15.63 pM solutions. Sample Diluent serves as the zero standard (0 pM).

Standard Point	Dilution	[rhTF] (pM)
P1	1 part Standard (1000 pM) + 1 part Sample Diluent	500.0
P2	1 part P1 + 1 part Sample Diluent	250.0
P3	1 part P2 + 1 part Sample Diluent	125.0
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	Sample Diluent	0.000

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.

Sample Preparation

✓ Plasma

Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes, use supernatants. Dilute samples 1:2 into Sample Diluent or within the range of 1x – 5x, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

✓ 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. Dilute samples 1:2 into Sample Diluent or within the range of 1x – 5x, and assay. 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 and assay. Store samples at -20°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-glucopyranoside at 37°C for 15 minutes. Collect fresh cell lysates and assay. The samples can be stored at -20°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 14000 x *g* for 20 minutes. 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°C.

Assay Procedure

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37°C for chromogenic activity assay. Seal the plate with sealing tape at each step.
2. Remove excess microplate strips from the plate frame.
3. Freshly prepare the desired volume of the Assay Mix by combining the following reagents according to the assay numbers (n) plus one.

Reagents	n=1
Assay Diluent	50 µL
FVII	10 µL
FX	10 µL

4. Add 70 µL of the above Assay Mix to each well of the 96-well plate.
5. Add 10 µL of rhTF Standard and sample per well of the 96-well plate. Mix gently
6. Incubate at 37°C for 30 minutes.
7. Add 20 µL of FXa Substrate to each well and mix gently. Read the absorbance at 405 nm at zero minutes for background O.D.
8. Seal the plate with sealing tape and incubate at 37°C and read the absorbance at 405 nm every 2 minute for 16 minutes.

Assay Mix	70 µL
TF Standard or Sample	10 µL
37°C, 30 minutes	
FXa Substrate	20 µL
Read the absorbance at 405 nm at zero minutes for background O.D. 37°C, Read the absorbance at 405 nm every 2 minutes for 20 minutes.	

✓ Summary

Add 70 µL of Assay Mix and add 10 µL of

Standard/sample per well

Incubate at 37°C for 30 minutes

⇩

Add 20 µL of Factor Xa Substrate per well

⇩

Read at 405 nm every 2 minutes for 16 minutes.

Data Analysis

Calculation of Results

- ✓ Calculate the mean value of the duplicate or 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 and multiply the value by the dilution factor.

Performance Characteristics

- ✓ The minimum detectable dose of TF is typically ~ 15.5 pM.
- ✓ This assay recognizes both natural and recombinant human TF.

Resources

References

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Plate Layout

12								
11								
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7								
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1								
	A	B	C	D	E	F	G	H