ab108906

Tissue Factor Activity Assay Kit (Human, Colorimetric)

Instructions for Use

For the quantitative measurement of Human Tissue Factor activity in plasma, serum, urine, cell lysates, and tissue samples

This product is for research use only and is not intended for diagnostic use.

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1. Introduction

The transmembrane protein Tissue factor (TF) is the physiologic trigger of coagulation in normal hemostasis. Tissue factor binds and allosterically activates factor VII (FVII). The TF-FVIIa complex cleaves factor IX and X, leading to thrombin generation. Tissue factor markedly enhances the ability of FVIIa to cleave both macromolecule and small peptidyl substrates. Inducible expression of Tissue factor in a variety of pathological conditions, including gram-negative sepsis and acute coronary syndromes, is associated with life-threatening thrombosis. In sepsis, Tissue factor expression within the vasculature leads to disseminated intravascular coagulation. Tissue factor also plays important roles in vasculogenesis, metastasis, and tumor-associated angiogenesis.

ab108906 Tissue Factor Activity Assay kit (Human, Colorimetric) is developed to determine Human Tissue Factor activity in plasma, serum, urine, cell lysates, and tissue samples. The kit is also validated for use with canine, bovine, equine, monkey, mouse, rat, swine, rabbit 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.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 70 µl Assay Mix to each well.

Add 10 µl Tissue Factor standard or samples to each well.
Incubate for 30 minutes at 37°C.

Add 20 µl FXa Substrate to each well. Incubate at 37°C. Read the absorbance at 405 nm every 5 minutes for 35 minutes. Cover and incubate at 37°C after each reading.

3. Kit Contents

- Tissue Factor Microplate: A 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.
- 1X Sample Diluent: 11 mL
- 1X Assay Diluent: 20 mL
- rh Tissue Factor Standard (lipoprotein) (lyophilized): 1 vial.
- Human FVII (lyophilized): 1 vial
- Human FX (lyophilized): 1 vial
- FXa Substrate (lyophilized): 2 vials

4. Storage and Handling

Store Standard, Factor VII protein, Factor X protein and FXa Substrate at -20°C. Store Microplate, Sample Diluent, Assay Diluent at 2-8°C. Opened Diluent may be stored for up to 1 month at 2-8°C.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 µL to 1 mL volumes.
- Distilled or deionized reagent grade water.
- Incubator at 37°C.

6. Preparation of Reagents

Sample Collection:

1. **Plasma:** Collect plasma using EDTA as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma. A human plasma sample is suggested for use at 1X, however, user should determine application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (Heparin can also be used as an anticoagulant, Sodium Citrate is not recommended).
2. **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. A human serum sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
3. **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes. A human urine sample is suggested for use at 1x or within the range of 2x – 5x into Sample Diluent; however, user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
4. **Cell Lysates:** The cultured cells are lysed and solubilized with 50 mM Tris-buffered saline (pH 8.0) containing 0.5% Triton X-100 or 0.1% Tween 20 on ice for 30 minutes. Centrifuge samples at 14000 rpm for 10 minutes. Collect fresh cell lysate. If necessary, dilute samples into Sample Diluent; however, user should determine optimal dilution factor depending on application needs. The

undiluted samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.

5. **Tissue:** Extract tissue samples with 50 mM Tris-buffered saline (pH 8.0) containing 0.5% Triton X-100 or 0.1% Tween 20 on ice for 30 minutes. Centrifuge samples at 14000 rpm for 20 minutes. Collect supernatant. If necessary, dilute samples into Sample Diluent; however, user should determine optimal dilution factor depending on application needs. Store remaining extract at -80°C. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

1. **Human FVII:** Add 1.4 ml reagent grade water to generate a 1X stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and used within 5 days.

2. **Human FX:** Add 1.4 ml reagent grade water to generate a 1X stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. Any remaining solution should be frozen at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.
3. **FXa Substrate:** Add 1.1 mL reagent grade water to generate a 1X stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C and used within 10 days.

4. **Standard Curve:** Standard Curve: Reconstitute the TF Standard with 1.5 mL reagent grade water to generate a solution of 500 pM. 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 (500 pM) 1:2 with Sample Diluent to produce 250, 125, 62.5, 31.25, 15.63, and 7.81 pM. Sample Diluent serves as the zero standard (0 pM).

   Any remaining TF Standard solution can be stored in single-use aliquots at -20°C for up to 5 days.

Standard Point	Dilution	[Tissue Factor] (pM)
P1	1 part Standard (500 pM) + 1 part Sample Diluent	250
P2	1 part P1 + 1 part Sample Diluent	125
P3	1 part P2 + 1 part Sample Diluent	62.5
P4	1 part P3 + 1 part Sample Diluent	31.25
P5	1 part P4 + 1 part Sample Diluent	15.63
P6	1 part P5 + 1 part Sample Diluent	7.81
P7	Sample Diluent	0.00

7. Assay Method

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 in a humid incubator to avoid evaporation.
2. Freshly prepare the desired volume of Assay Mix by combining the following reagents as described below according to the number of wells in the assay (n) plus one well. The values represent the volumes for one well:

Assay Diluent	50 µL
Human FVII	10 µL
Human FX	10 µL

3. Add 70 µL of the Assay Mix to each well.
4. Add 10 µL of Tissue Factor Standard or sample to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes at 37°C in a humid incubator.
5. Add 20 µL of FXa Substrate to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate at 37°C in a humid incubator for 5 minutes.
6. Read absorbance at 405 nm and carry out a reading every 5 minutes for 30 minutes. Cover wells with sealing tape and incubate at 37°C after each reading.

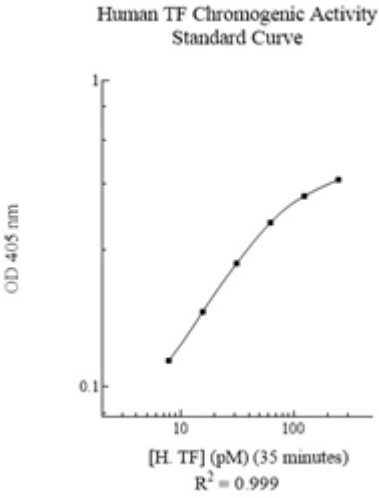
8. Data Analysis

Calculate the mean value of the triplicate readings for each standard and sample. Determine the optimal time frame in which the various standard concentrations have steady OD increase, and use this optimal time frame data for standard curve plotting and data analysis. Determine ΔOD/min, ie total OD increase of sample over total time frame and divide by number of minutes/time frame. Plot the standard curve (x-axis: TF concentration; y-axis: OD405) using standard readings calculated above. Determine the best-fit line by regression analysis of the 4 parameter curve. Interpolate the sample concentration using the sample readings from the linear part of the standard curve

A. Typical Data

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

This assay recognizes both natural and recombinant Human Tissue Factor.



B. Sensitivity

The minimum detectable dose of Tissue Factor at 30 minutes is typically 3.5 pM.

9. Troubleshooting

Problem	Cause	Solution
Poor standard curve	Improper standard dilution	Confirm dilutions made correctly
	Standard improperly reconstituted (if applicable)	Briefly spin vial before opening; thoroughly resuspend powder (if applicable)
	Standard degraded	Store sample as recommended
	Curve doesn't fit scale	Try plotting using different scale
Low signal	Incubation time too short	Try overnight incubation at 4°C
	Target present below detection limits of assay	Decrease dilution factor; concentrate samples
	Precipitate can form in wells upon substrate addition when concentration of target is too high	Increase dilution factor of sample
	Using incompatible sample type (e.g. serum vs. cell extract)	Detection may be reduced or absent in untested sample types
	Sample prepared incorrectly	Ensure proper sample preparation/dilution

Problem	Cause	Solution
High background	Wells are insufficiently washed	Wash wells as per protocol recommendations
	Contaminated wash buffer	Make fresh wash buffer
	Waiting too long to read plate after adding STOP solution	Read plate immediately after adding STOP solution
Large CV	Bubbles in wells	Ensure no bubbles present prior to reading plate
	All wells not washed equally/thoroughly	Check that all ports of plate washer are unobstructed/wash wells as recommended
	Incomplete reagent mixing	Ensure all reagents/master mixes are mixed thoroughly
	Inconsistent pipetting	Use calibrated pipettes and ensure accurate pipetting
	Inconsistent sample preparation or storage	Ensure consistent sample preparation and optimal sample storage conditions (eg. minimize freeze/thaws cycles)
Low sensitivity	Improper storage of ELISA kit	Store all reagents as recommended. Please note all reagents may not have identical storage requirements.
	Using incompatible sample type (e.g. Serum vs. cell extract)	Detection may be reduced or absent in untested sample types

Technical Support

For all technical or commercial enquiries please go to:

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