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ACTICHROME® TF

REF 846

for Research Use Only



INTENDED USE

The ACTICHROME® TF activity assay is a chromogenic assay intended for the measurement of human tissue factor procoagulant activity in cell lysates and human plasma. This assay is intended for research use only. It is not intended for diagnostic or therapeutic use.

EXPLANATION OF THE TEST

Tissue Factor (TF) is a 45 kD transmembrane cell surface glycoprotein known for its role in initiating coagulation for the past 90 years.[1] TF is comprised of three domains: an extracellular domain (aa 1-219), followed by a hydrophilic spanning domain (aa 220-242) and a cytoplasmic tail (aa 243-263).[2] It functions as a receptor and cofactor for the latent serine proteases factor VII and VIIa.[3,4] Contact between TF and blood is sufficient to initiate the extrinsic pathway of coagulation. TF is located on the cells in the adventitia and variably on cells in cell culture.

In vitro studies reveal that once TF complexes with factor VII, factor VII is efficiently activated by factor Xa. Other proteases including factor IXa, factor XIIa and thrombin are also capable of activating factor VII. As with all vitamin K-dependent zymogens, activation requires the presence of calcium ions and phospholipids. Factor VII activation differs from the activation of other vitamin K-dependent zymogens by its binding to TF, which causes a major allosteric change, and its upregulation by TF. Formation of this TF/FVIIa complex renders the factor VII bond at Arg152 - Ile153 susceptible to cleavage by trace amounts of factor Xa and factor IXa. Activation by factor Xa is profoundly enhanced by lipidated TF but not by soluble TF (aa 1-219). TF lipidation by acidic phospholipids is more efficient than vesicles made with phosphatidylcholine (PC). This suggests that factor Xa mediated activation of factor VII requires binding of the Gla-domain to the phospholipid head groups.[5,6]

Factor VIIa possesses little proteolytic activity in itself. Only when bound to TF does factor VIIa possess sufficient proteolytic activity to activate factor IX and factor X. Although factor VIIa possesses some activity towards peptidyl substrates in the presence of apo TF protein and calcium ions, this is not the case for physiological substrates such as factor IX and factor X. These factors require negatively charged phospholipids for full expression of factor VIIa proteolytic activity.[7] Gamma carboxylation of the factor VII Gla-domain is crucial for manifestation of full biologic activity. Factor VII mutants lacking the Gla-domain have a markedly diminished capacity to bind to tissue factor. The EGF domain of factor VIIa (aa 51-88) is possibly a TF binding site. The interaction of factor VIIa with TF involves multiple binding sites: the N-terminal Gla-domain, the EGF domain, and amino acid residues located in the factor VII protease domain.

When monocytes and macrophages are stimulated by endotoxins, cytokines and lectins, TF is upregulated in these cells with an increase in procoagulant activity (PCA). Studies have reported patients diagnosed with Disseminated Intravascular Coagulation (DIC) as having an increase in TF plasma levels.[10]

Tissue Factor is released into the blood stream following disruption of the endothelium. The initiation of the coagulation pathways requires the participation of a series of molecules; TF and its ability to complex with, factor VII[11], factor X or factor IX, charged phospholipids and calcium (the catalytic activity of factor VIIa in comparison to VII is insignificant).[12] The TF/FVIIa complex efficiently activates both factor X and factor IX, thus initiating both the intrinsic and extrinsic coagulation pathways.[13] The extrinsic pathway is blocked by Tissue Factor Pathway Inhibitor, TFPI, the only effective inhibitor of TF/FVIIa allowing factor IX to activate factor X.[14]

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PRINCIPLE OF THE METHOD

The ACTICHROME TF assay measures the peptidyl activity of human tissue factor in cell lysates and human plasma. Samples are mixed with human factor VIIa and human factor X and incubated at 37°C, during which time the tissue factor/factor VIIa complex (TF/FVIIa) is formed and the complex converts the human factor X to Factor Xa. The amount of factor Xa generated is measured by its ability to cleave SPECTROZYME® FXa, a highly specific chromogenic substrate for factor Xa, which is added to the reaction solution. The cleaved substrate releases a para-nitroaniline (pNA) chromophore into the reaction solution. The solution absorbance is read at 405 nm and compared to those values obtained from a standard curve generated using known amounts of human tissue factor.

REAGENTS

ACTICHROME TF contains sufficient reagents to perform 100 tests using a microtiter well format; 44 samples assayed in duplicate.

1. Assay Buffer: 1 vial of 5 mL, 10X concentrate
2. Human Factor VIIa: 2 vials (lyophilized)
3. Human Factor X: 2 vials (lyophilized)
4. SPECTROZYME® FXa: 2 vials, 5 µmoles (lyophilized)
5. TF/TFPI Depleted Plasma: 2 vials, 0.5 mL (lyophilized)
6. Relipidated Human Tissue Factor: 1 vial, 500 pM (lyophilized)

WARNINGS AND PRECAUTIONS

The TF/TFPI Depleted Plasma provided is of human origin. Each donor unit used in the manufacture of this reagent has been tested and found to be non-reactive for Hepatitis B surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus (HIV). As no known method can offer complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV or other blood-borne pathogens, this plasma should be handled as recommended for any potentially infectious human serum or blood specimen.

Do not use kit components beyond the expiration date. Do not mix reagents from different kits. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.

Assay Buffer	Warning		CONT: Tris-(hydroxymethyl)aminomethane H315, H319, P264, P280, P302 + P352, P305 + P351 + P338, P332 + P313, P337 + P313
Factor VIIa	Warning		CONT: 2-Amino-2-(hydroxymethyl)propane-1,3-diol hydrochloride H315, H319, H335, P261, P264, P280, P302 + P352, P304 + P340, P305 + P351 + P338, P332 + P313, P337 + P313
Factor X	Warning		H315, H319, H335, P261, P264, P280, P302 + P352, P304 + P340, P305 + P351 + P338, P332 + P313, P337 + P313
Spectrozyme FXa	Warning		CONT: N-Methoxycarbonyl-D-cyclohexylglycyl-glycyl-arginine-para-nitroanilide acetate H315, H319, H335, P261, P264, P280, P302 + P352, P304 + P340, P305 + P351 + P338, P332 + P313, P337 + P313
Relipidated Tissue Factor	Warning		H315, H319, H335, P261, P264, P280, P302 + P352, P304 + P340, P305 + P351 + P338, P332 + P313, P337 + P313

Hazard Statements:
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H335 May cause respiratory irritation.

Precautionary Statements:
P261 Avoid breathing dust.
P264 Wash thoroughly after handling.
P280 Wear protective gloves/eye protection/face protection.
P302 + P352 If on skin: Wash with plenty of water.
P332 + P313 If skin irritation occurs: Get medical advice/attention.
P304 + P340 If inhaled: Remove person to fresh air and keep comfortable for breathing.
P305 + P351 + P338 If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313 If eye irritation persists: Get medical advice/attention.

REAGENT RECONSTITUTION AND STABILITY

Unopened reagents are stable until the expiration date indicated on their label when stored at 2° - 8°C. Reconstituted reagents may be aliquoted and stored at -20°C for up to one month. Do not submit frozen reagents to multiple freeze-thaw cycles.

1. **Assay Buffer**: Add the contents of the vial (5 mL) to 45 mL of cold filtered deionized water and mix thoroughly.
2. **Human Factor VIIa:** Add 1.4 mL of filtered deionized water to the vial.
3. **Human Factor X**: Add 1.4 mL of filtered deionized water to the vial.
4. **SPECTROZYME® FXa**: Add 2.0 mL of filtered deionized water to the vial and mix thoroughly. Reconstituted substrate may be stored at -20°C or colder for up to 6 months.
5. **TF/TFPI Depleted Plasma**: Add 0.5 mL of Assay Buffer to the vial, mix thoroughly and place the vial on ice for 3 minutes. Add the content of the vial to an additional 9.5 mL of Assay Buffer (a 1:20 dilution) to create a 5% TF/TFPI Depleted Plasma. Place on ice for immediate use or aliquot into plastic cryotubes and store **immediately** at -20°C.

6a. **Tissue Factor Standard for Cell Lysate Samples**: Add 2.3 mL of Assay Buffer to the vial of Relipidated Human Tissue Factor to generate a 500 pM solution. Aliquot and immediately freeze at –20°C the stock 500 pM Tissue Factor solution that will not be used immediately. Label cryotubes and prepare standards of Tissue Factor as listed below in Table 1, by adding the specified volume of Standard to the specified volume of Assay Buffer. Discard any unused diluted standards.

6b. **Tissue Factor Standard for Plasma Samples**: Add 2.3 mL of 5% TFPI Depleted Plasma to the vial of Relipidated Human Tissue Factor to generate a 500 pM solution. Label cryotubes and prepare standards of Tissue Factor as listed below in Table 1, by adding the specified volume of Standard to the specified volume of 5% TFPI Depleted Plasma. Discard any unused diluted standards.

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Table 1 – Preparation of Tissue Factor Standard

Tissue Factor Standard Concentration	Volume of Tissue Factor Standard	Volume of Assay Buffer or 5% TF/TFPI Depleted Plasma
30 pM	20 µL of 500 pM	313 µL
15 pM	100 µL of 30 pM	100 µL
7.5 pM	100 µL of 15 pM	100 µL
3.75 pM	100 µL of 7.5 pM	100 µL
1.88 pM	100 µL of 3.75 pM	100 µL
0.0 pM	0 µL	100 µL

SPECIMEN COLLECTION AND PREPARATION

A. Cell Lysates

Cells may be lysed by repeated freeze-thaws and/or sonication in a buffer of 50 mM Tris-HCl, 100 mM NaCl, 0.1% Triton X-100, pH 7.4, and the tissue factor extracted in the buffer for 30 minutes at 37°C or for 18 hours at 2°-8°C. Typically, samples are assayed neat but dilute with Assay Buffer if necessary. Store extracted lysates at -70°C.

B. Plasma

Only citrate collected platelet poor plasma may be used for this assay. Do Not Use EDTA collected plasma. See "*Collection, Transport and Processing of Blood Specimens for Testing Plasma-based Coagulation Assays and Molecular Hemostasis Assays; Approved Guidelines-Fifth Edition*", CLSI Document H21-A5, Vol. 28, No. 5, January 2008. Plasma collection should be performed as follows:

1. Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
2. Centrifuge the blood sample at 1,500 x g for 15 minutes.
3. Plasma should be stored at room temperature and assayed within 2 hours. Alternatively, plasma may be stored at –70°C for up to 6 months.
4. Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at room temperature and assayed within 2 hours.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided
96 well round bottom microtiter plate
0.22 µm filtered deionized H_20
200-1000 µL single pipette, 10-100 µL single pipette
5-50 µL eight channel multi-pipette
Glacial acetic acid
Microtiter plate reader at 405 nm

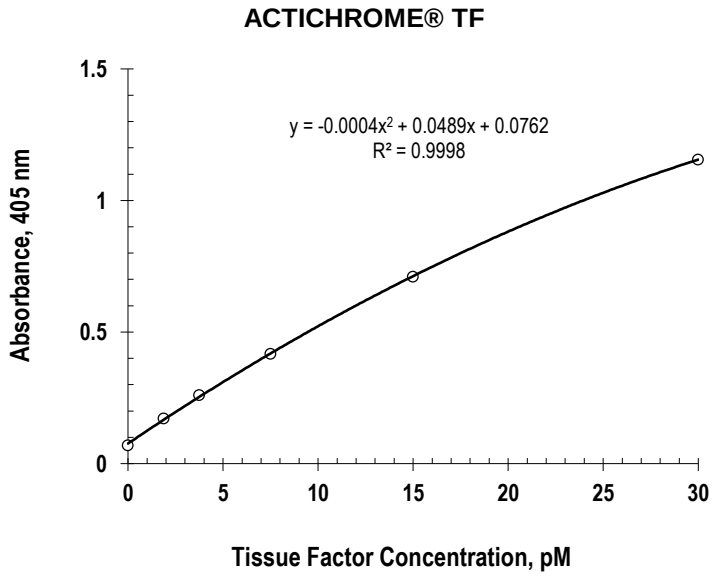
Assay Procedure

1. Add 50 µL Assay Buffer (pH 8.4) to each well.
2. Add 25 µL Tissue Factor Standards or test sample to each well.
3. Add 25 µL of Human Factor VIIa to each well.
4. Add 25 µL of Human Factor X. Cover the microwells and incubate at 37°C for 15 minutes.
5. Add 25 µL of SPECTROZYME FXa substrate to each well and incubate at 37°C for 30 minutes. The enzyme/substrate reaction begins upon addition of the SPECTROZYME FXa, turning the solution yellow over time.
6. Stop the reaction after the 30 minutes by adding 50 µL of glacial acetic acid. Read the absorbance of the solution at a wavelength of 405 nm. For plasma samples, read the solution absorbance at 405 nm and at 490 nm. Use the $\Delta A_{405-490}$ of the sample for interpolating the tissue factor concentration.

RESULTS

Representative Standard Curve

The standard curve is constructed by plotting the mean absorbance value measured for each Tissue Factor Standard versus its corresponding concentration. A standard curve should be constructed each time the assay is performed. The following curve has been plotted using a 2nd order polynomial regression and is for demonstration purposes only.



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CALCULATION

Interpolate the Tissue Factor concentration of the test sample directly from the standard curve. If the sample has been diluted, multiply the results of the sample by the dilution factor to obtain its actual tissue factor concentration.

ANALYTICAL PERFORMANCE

Specificity: The assay recognizes native and recombinant human relipidated tissue factor. No interference from other coagulation factors has been observed.

Sensitivity: The lower limit of detection for plasma samples has been found to be approximately 2 pM (approximately 85 pg/mL), determined by adding two standard deviations to the mean OD value of the "0" standard (n=10) and interpolating the corresponding concentration from the standard curve.

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DEFINITION OF SYMBOLS

	Consult instructions for use
	Refer to Safety Data Sheet
	Manufactured by
	Store at 2°C to 8°C
LOT	Batch code / Lot number
	Expiration Date
REF	Catalog number
	Contains sufficient for <n> tests
CONT	Contains...

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