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ACTICHROME® TFPI

REF 848

Chromogenic Assay for Measuring TFPI Activity in Human Plasma

For Research Use Only

INTENDED USE

The ACTICHROME® TFPI is a chromogenic assay intended for the measurement of Tissue Factor Pathway Inhibitor (EPI, LACI)[1] activity in human plasma where TFPI exhibits an inhibitory effect on the Tissue Factor/FVIIa complex. This assay is intended for research use only. It is not intended for use in diagnostic or therapeutic use.

EXPLANATION OF THE TEST

TFPI circulates in human plasma as complexes with LDL, HDL and VLDL[2] and can be found in several forms: a 36,000 D molecule, a 43,000 D molecule and as truncated moieties. This heterogeneity of size appears, in part, to be the result of the formation of mixed disulfide complexes between TFPI and apolipoprotein AII.[3] In humans, approximately 10% of total TFPI is carried by platelets which release TFPI once they are activated by thrombin.[4] Thus, at the site of a wound, where platelets aggregate, elevated levels of TFPI are present. Based on the initial isolation of the inhibitor[5] it was found that TFPI inhibits Tissue Factor (TF) procoagulant activity; i.e., the TF/FVIIa complex, and directly inhibits factor Xa by binding at or near its serine active site.[6]

The inhibitory mechanism of TFPI is a two-step process. In the first step TFPI binds to factor Xa via its Kunitz-2 domain, followed by a second step in which the TFPI/FXa complex binds to the TF/FVIIa complex via its Kunitz-1 domain, forming an inactive quaternary TFPI/FXa/TF/FVIIa complex. The direct inhibition of factor Xa is based on a 1:1 stoichiometry and is not calcium dependent.[6] Furthermore, factor Xa inhibition does not solely rely on TFPI binding through Kunitz-2 domain.

The C-terminal region of TFPI is required for a high affinity binding between TFPI and factor Xa and the subsequent factor Xa inhibition.[7] It has been found that TFPI is released into blood following administration of heparin and that heparin enhances TFPI inhibition of factor Xa, and that the C-terminal region is the major heparin binding site.[8]

PRINCIPLE OF THE METHOD

The ACTICHROME TFPI Activity Assay measures the ability of TFPI to inhibit the catalytic activity of the TF/FVIIa complex to activate factor X to factor Xa. After incubation of test samples with TF/FVIIa and FX, the residual activity of the TF/FVIIa complex is measured using SPECTROZYME® FXa, a highly specific chromogenic substrate cleaved only by FXa generated in the assay, releasing a p-nitroaniline (pNA) chromophore. The absorbance of the pNA in the reaction solution at 405 nm is measured and compared to those values obtained from a standard curve constructed using known TFPI activity levels. This assay may be performed in either end-point or kinetic mode.

REAGENTS

This kit contains reagents sufficient to perform 100 test points.

1 vial of Assay Buffer, 5 mL (5X concentrate)
1 vial of SPECTROZYME® FXa, 5 µmoles (lyophilized)
2 vials of TFPI Depleted Plasma, 0.5 mL (lyophilized)
1 vial of TFPI Reference Plasma, 0.5 mL, ca. 1 unit/mL (lyophilized)
1 vial of Human Factor X, 25 µg (lyophilized)
1 vial of Relipidated Tissue Factor, 50 ng (lyophilized)
1 vial of Human Factor VIIa Reagent (lyophilized)
1 vial of TFPI Standard, 0.2 unit/mL (lyophilized)

WARNINGS AND PRECAUTIONS

Source material for some of the reagents in this kit is of human origin. This material has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV-2) using FDA approved methods. As no known test method provides complete assurance that products derived from human blood will not transmit HBsAg, HCV, HIV-1, HIV-2 or other blood-borne pathogens, reagents should be handled as recommended for any potentially infectious human specimen. Discard all waste associated with test specimens and human source reagents in a biohazard waste container.

Do not use kit components beyond the expiration date. Do not mix reagents from different kit lots. Avoid microbial contamination of the kit components. Do not mouth pipette or ingest reagents.

Assay Buffer	Warning		H315, H319, H335, P264, P280, P302 + P352, P332 + P313, P305 + P351 + P338, P337 + P313
SPECTROZYME® FXa	Warning		CONT: N-Methoxycarbonyl-D-cyclohexylglycyl-glycyl-arginine-para-nitroanilide acetate H315, H319, H335, P261, P264, P280, P302 + P352, P305 + P351 + P338, P332 + P313, P337 + P313
Human Factor X Reagent	Warning		H315, H319, H335, P261, P264, P280, P302 + P352, P332 + P313, P305 + P351 + P338, P337 + P313
Relipidated Tissue Factor	Warning		H315, H319, H335, P261, P264, P280, P302 + P352, P332 + P313, P305 + P351 + P338, P337 + P313
Human Factor VIIa Reagent	Warning		H315, H319, H335, P261, P264, P280, P302 + P352, P332 + P313, P305 + P351 + P338, 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/ protective clothing/ eye protection/ face protection.
P302 + P352 IF ON SKIN: Wash with plenty of water.
P305 + P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P332 + P313 If skin irritation persists: Get medical advice/attention.
P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.
P337 + P313 If eye irritation persists: Get medical advice/attention.

REAGENT PREPARATION AND STORAGE

Unopened and lyophilized reagents are stable until the expiration date indicated on the label when stored at 2°C to 8°C.

1. **Assay Buffer:** Add the contents of the vial to 20 mL of cold (2°-8°C) filtered deionized water and mix thoroughly.
2. **SPECTROZYME FXa:** Add 2.1 mL of filtered deionized water to the vial and mix thoroughly. Reconstituted material may be stored at -20°C or colder for up to one year.
3. **TFPI Depleted Plasma:** Add 0.5 mL of Assay Buffer to each vial of TFPI Depleted Plasma, mix thoroughly and place on melting ice. Combine the contents of these vials and add to 19 mL of Assay Buffer. Aliquot this 5% TFPI Depleted Plasma into labeled plastic cryotubes, placing aliquots for immediate use on melting ice. Store unused aliquots immediately at -20°C for up to one month.
4. **TFPI Reference Plasma:** Add 0.5 mL of filtered deionized water to the vial of TFPI Reference Plasma, mix thoroughly and place the vial on melting ice for 3 minutes. Aliquot into labeled plastic cryotubes. Place aliquots for immediate use on melting ice. Store unused aliquots immediately at -20°C for up to one month.
5. **Human Factor X:** Add 2.5 mL of filtered distilled water to the vial of Human Factor X and mix thoroughly. Aliquot into labeled plastic cryotubes. Place aliquots for immediate use on melting ice. Store unused aliquots immediately at -20°C for up to one month.
6. **Relipidated Tissue Factor:** Add the volume of Assay Buffer specified on the label to the vial of relipidated Tissue Factor and mix thoroughly. Aliquot into labeled plastic cryotubes. Place aliquots for immediate use on melting ice. Store unused aliquots immediately at -20°C for up to one month.
7. **Human Factor VIIa Reagent:** Add 2.25 mL of filtered deionized water to the vial of Human Factor VIIa reagent and mix thoroughly. Aliquot into labeled plastic cryotubes. Place aliquots for immediate use on melting ice. Store unused aliquots immediately at -20° for up to one month.

8. **TFPI Standard:** Add 1.0 mL of filtered deionized water to the TFPI Standard vial to generate a 0.2 unit/mL standard. Prepare TFPI Standard Concentrations of 0.2, 0.1, 0.08, 0.06, 0.04 and 0.02 unit/mL by diluting the 0.2 unit/mL TFPI Standard as shown in **Table 1**. Label cryotubes and aliquot accordingly. Store unused standards immediately at -20°C for up to one month.

Table 1 - TFPI Standard Dilutions

TFPI Standard Concentration, unit/mL	Volume of 0.2 unit/mL Standard	Volume of TFPI Depleted Plasma
0.20	As needed	0 µL
0.10	100 µL	100 µL
0.08	80 µL	120 µL
0.06	60 µL	140 µL
0.04	40 µL	160 µL
0.02	20 µL	180 µL
0	0 µL	As needed

9. **Tissue Factor/FVIIa Reagent:** Add 26.6 µL of Relipidated Tissue Factor per mL of Human Factor VIIa. Prepare the TF/FVIIa reagent fresh each time the assay is run. Discard any unused factor TF/FVIIa reagent.

10. **EDTA:** Prepare a solution of EDTA as follows: Dissolve 48 mg of EDTA (trisodium, dihydrate) in 2.0 mL of filtered deionized water. Mix thoroughly, adjust the pH to 9.9 with NaOH and q.s. to 2.5 mL with filtered deioinzed H_2O.

SPECIMEN COLLECTION AND PREPARATION

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, Juanuary 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 10,000 x g for 15 minutes.
3. Plasma should be stored at room temperature and assayed within 2 hours. Alternatively, plasma may be stored at –80°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.

Dilute the TFPI Reference Plasma and each test sample 1:20 (i.e., add 15 µL reference plasma/test sample to 285 µL TFPI Depleted Plasma) prior to performing the assay.

PROCEDURE

Materials Provided – See Reagents

Material Required But Not Provided

EDTA (trisodium, dihydrate)
1 x 96 microwell test plate (round bottom)
0.22 µm filtered deionized water
200 - 1000 µL single pipette
10 - 100 µL single pipette
50 - 100 µL eight channel multi-pipette
Microwell plate reader at 405 nm
Glacial Acetic Acid

Assay Procedure

Microwell End-Point Method

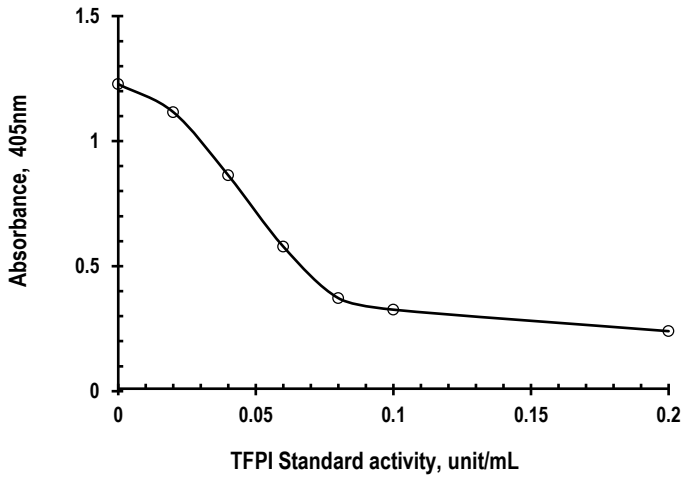
1. Add 20 µL of TFPI Standard, diluted Reference Plasma or diluted test sample to a microwell.
2. Add 20 µL of TF/FVIIa complex to the microwell.
3. Cover the microwell and incubate at 37°C for 30 minutes.
4. Add 20 µL of Human Factor X to each microwell.
5. Cover and incubate at 37°C for 15 minutes.
6. Add 20 µL of EDTA to each microwell.
7. Add 20 µL of SPECTROZYME FXa substrate to each microwell.
8. The reaction will begin immediately upon addition of the SPECTROZYME FXa and the solution will turn yellow as the reaction continues. Stop the reaction at 5 minutes by adding 50 µL of glacial acetic acid to each well. Read the absorbance of the solution at a wavelength of 405 nm.

RESULTS

Representative Standard Curve

The standard curve is constructed by plotting the mean absorbance value calculated for each TFPI standard at 405 nm versus the corresponding concentration. A standard curve should be generated each time the assay is performed. The following curve is for demonstration purposes only and depicts a spline interpolation regression analysis. The laboratory should select the best curve fit (i.e. spline vs. polynomial) for the data generated.

ACTICHROME® TFPI Activity Assay



CALCULATIONS

Interpolate the TFPI concentrations for the diluted samples directly from the standard curve. Multiply the results of the tested samples and the Reference Plasma by the dilution factor of 20 to obtain the actual TFPI concentration of the sample. If a higher or lower dilution has been used, multiply the final result by the appropriate dilution factor.

LIMITATIONS OF PROCEDURE

The kit reagents contain agents that neutralize heparin up to and including 5 units/mL. Testing on plasmas containing higher heparin levels may produce inaccurate results.

REFERENCES

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DEFINITION OF SYMBOLS

	Consult instructions for use	LOT	Lot number
	Refer to SDS		Expiration Date
IVD	In vitro diagnostic medical device	REF	Catalog number
	Manufactured by	Σ n	Contains sufficient for <n> tests
2°C 8°C	Store at 2°C to 8°C	CONT	contains