

ACTICHROME® FX

Product No. 880

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INTENDED USE

ACTICHROME® FX is a chromogenic assay for the measurement of factor X activity in human plasma. This product is limited for Research Use Only.

PRINCIPLE OF THE METHOD

The method is based upon a two-stage reaction. In stage one, factor X in the plasma sample is activated to factor Xa by Russell's Viper Venom (RVV) in the presence of calcium. In stage two, the factor Xa generated hydrolyzes the Spectrozyme® FXa, a chromogenic substrate releasing the chromophore, pNA. The color of the reaction solution is read spectrophotometrically at 405 nm. The absorbance of the solution is directly proportional to the amount of FXa activity in the sample.

REAGENTS

5 vials of Spectrozyme® FXa, 5 µmoles (lyophilized)
5 vials of Russell's Viper Venom, 100 µg (lyophilized)
1 vial of 0.1 M CaCl₂, 4 mL
3 vials of 0.05M Tris, pH 8.4 with 0.002% Polybrene®, 20 mL

REAGENT PREPARATION AND STORAGE

- Spectrozyme® FXa:** Reconstitute with 1.0 mL of filtered deionized water to obtain a concentration of 5 mM. The reconstituted substrate is stable for up to 1 month at 2°-8°C and for up to 6 months at -20°C.
- Russell's Viper Venom:** Reconstitute with 0.5 mL of filtered deionized water. The reconstituted activator is stable for 1 month at -20°C.
- CaCl₂:** Supplied ready to use, once opened, the calcium chloride may be used for up to 4 weeks when stored at 2°-8°C.
- Tris:** Supplied ready to use, once opened, the Tris buffer may be used for up to 4 weeks when stored at 2°-8°C.
- RVV + CaCl₂** – Just prior to use, mix equal volumes of RVV and CaCl₂. The mixture is stable for 48 hours at 2°-8°C and for 4 months at -20°C.

SPECIMEN COLLECTION

Citrate collected platelet poor plasma may be used for this assay. See "Collection, Transport and Preparation of Blood Specimens for Coagulation Testing and Performance of Coagulation Assays", NCCLS Document H21-A4, Vol. 23, No. 35, December 2005. Plasma collection should be performed as follows:

- Collect 9 parts of blood into 1 part of 3.2% (0.109 M) trisodium citrate anticoagulant solution.
- Centrifuge the blood sample at 1,500 x g for 15 minutes.
- Plasma should be stored at 2°-8°C and assayed within 4 hours. Alternatively, plasma may be stored at -20°C for up to 6 months.
- Frozen plasma should be thawed rapidly at 37°C. Thawed plasmas should be stored at 2°-8°C and assayed within 4 hours.

PROCEDURE

Materials Provided – See Reagents

Materials Required But Not Provided

96 microwell round bottom plate
Pooled Normal Plasma (eg. REF 258N)
0.22 µm filtered deionized water
20% acetic acid
microwell plate reader set at a wavelength of 405 nm
8-channel pipette covering 50 - 200 µL
1-channel pipettes covering 10 - 1000 µL
37°C water or dry bath
laboratory timer

Assay Calibration

A standard curve is required for each test series and is generated using a pooled normal plasma from at least 20 normal donors that has been collected in the same manner as the plasmas to be tested.

The following standards should be prepared.

FX Standard	Volume of PNP	Volume of Buffer
100%	100 µL	0 µL
75%	75 µL	25 µL
50%	50 µL	50 µL
25%	25 µL	75 µL
10%	10 µL	90 µL
5%	5 µL	95 µL
0%	0 µL	100 µL

Assay Procedure

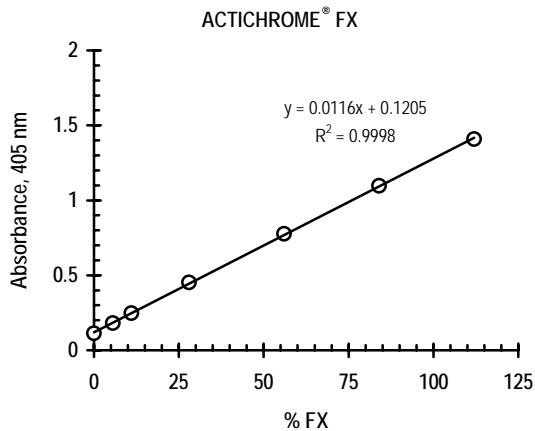
FX Standards, controls and plasma samples are diluted 1:20 (25 µL of plasma + 475 µL) with the Tris buffer.

- Add 50 µL of diluted standard, control or plasma sample to a microwell.
- Incubate at 37°C for 4 minutes.
- Add 50 µL of RVV + CaCl₂ mixture.
- Incubate at 37° for 2 minutes.
- Add 50 µL of 5 mM Spectrozyme FXa
- Incubate at 37°C for exactly 10 minutes.
- Stop the reaction by adding 50 µL of 20% acetic acid and measure the absorbances at 405 nm using a microwell plate reader.

RESULTS

Representative Standard Curve

For assaying via an endpoint method, construct a standard curve by plotting the mean absorbance value for each factor X standard versus its corresponding concentration in percent (%). The % FX in the unknown plasma sample can be determined by direct interpolation from the standard curve. A calibration curve should be generated each time the assay is performed. The curve shown below are for example only.



EXPECTED VALUES

In healthy, normal subjects, the physiological level of Factor X ranges from 60-120%. In those patients receiving anticoagulant therapy, the range of Factor X is 15-30%.

PERFORMANCE CHARACTERISTICS

Lower Limit of Detection

The assay allows detection of factor X down to as low as 5%. However, at concentrations below 25%, it is recommended that the unknown plasma be diluted 1:10 (100 μ L of plasma + 900 μ L of Tris Buffer) and the measured concentration divided by a factor of 2.

Sensitivity

The assay is linear in the 5-100% range of normal plasma.

Interfering Substances

Antithrombin concentrations up to 5 times the normal level in plasma do not influence the assay.

Heparin concentrations below 30 Units/mL are neutralized by the Polybrene in the Tris buffer and do not influence the assay.

Bilirubin concentrations above 750 μ M, hemoglobin concentrations above 450 μ M and plasma from hyperlipemic patients may present difficulties in measuring the absorbance.

REFERENCES

1. Kiesel, E., *et al.* Factor X activating enzyme from Russell's Viper Venom; Isolation and characterization. *Biochemistry* 1976, 15: 4901-4906.
2. Aurell, *et al.* A new sensitive and highly specific chromogenic peptide substrate for Factor Xa. *Thrombosis Research* 1977, 11: 595-605.
3. Lindhout, M. J. *et al.* Activation of decarboxyfactor X by a protein from Russell's Viper Venom. Purification and partial characterization of activated decarboxyfactor X. *Biochem Biophys Acta.* 1978, 533: 327-341.
4. Bergstrom, K. and Egberg, N. Determination of vitamin K sensitive coagulation factors in plasma. Studies on three methods using synthetic chromogenic substrates. *Thrombosis Research* 1978, 12: 531-547.