

# DIAPHARMA FACTOR X KIT

KDPGFX

For *in vitro* diagnostic use

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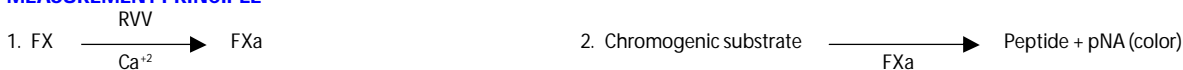
## INTENDED USE

This kit is for the quantitative determination of Factor X activity in human citrated plasma. The DiaPharma Factor X Kit is an *in vitro* diagnostic test kit for the quantitative determination of Factor X activity in human citrated plasma. Factor X activity is useful for monitoring patients on oral anticoagulant therapy (warfarin) where baseline INR values are prolonged and INR results are not reliable, such as in OAC patients with lupus inhibitors. The DiaPharma Factor X Kit is also useful for screening for factor X deficiencies.

## SUMMARY AND EXPLANATION

Coagulation Factor X (Stuart Prower Factor, FX) is a vitamin K dependent protein produced by the liver. It has a central position in the coagulation cascade. Factor X is activated both by the extrinsic and intrinsic systems before exerting its effect on the conversion of prothrombin to thrombin. Patients who need anticoagulation therapy can be treated with warfarin, which is a vitamin K antagonist. This treatment leads to a decrease in all vitamin K dependent clotting factors and thus a prolonged clotting time of blood. The therapy requires careful monitoring since it is essential to find an optimal balance between risk of thrombosis and risk of bleeding for each patient. The DiaPharma Factor X kit is a useful tool in the management of patients with lupus inhibitors receiving warfarin therapy.

## MEASUREMENT PRINCIPLE



The method is based on a two-stage principle. In stage one, Factor X is activated in the presence of calcium to Factor Xa (FXa) by the activator Russell's Viper Venom (RVV). In stage two, the generated FXa hydrolyses the chromogenic substrate, thus liberating the chromophoric group, pNA. The color is then read with a spectrophotometer at 405 nm. The generated FXa and thus the intensity of color is proportional to the FX activity in the sample.

## REAGENTS

### • Chromogenic Substrate

*Preparation* 25 mg lyophilized FXa chromogenic substrate with mannitol added as a bulking agent. Reconstitute substrate with 20 ml sterile water.  
*Storage & Stability* The reconstituted substrate is stable for 6 months at 2 - 8°C.

### • Russell's Viper Venom

*Preparation* FX activating protein from Russell's Viper Venom. Reconstitute the RVV with 15 ml sterile water.  
*Storage & Stability* The reconstituted activator is stable for 1 month at 2 - 8°C.

### • CaCl<sub>2</sub>

*Preparation* 20 ml of 0.1 mol/L calcium chloride solution. Before use, mix 1 volume of RVV with 1 volume of CaCl<sub>2</sub>. The mixture is stable for 48 hours at 2 - 8°C.  
*Storage & Stability* The solution is stable at 2 - 8°C until the expiry date printed on the label.

### • Buffer

*Preparation* 100 ml buffer solution containing 0.05 mol/L Tris, pH 7.8 and 20 mg/L Polybrene® (hexadimethrine bromide). Ready for use.  
*Storage & Stability* The buffer is stable at 2 - 8°C until the expiry date printed on the label.

The sealed reagents are stable at 2 - 8°C until the expiry date printed on the label. Contamination by microorganisms should be avoided once the vials are opened.

## Materials required but not provided

- Sterile water
- Acetic acid, 20% (acid-stopped method only)
- Spectrophotometer, 405 nm
- Microplates if needed
- Microplate reader if needed
- Incubator 37° ± 0.2°C
- Vortex mixer
- Centrifuge, 2000 x g
- Calibrated pipettes
- Calibration plasma
- Control plasma

## QUALITY CONTROLS

Normal and abnormal controls are recommended for a complete quality control program. Each laboratory should establish its own mean and standard deviation and should establish a quality controls program to monitor laboratory testing. Controls should be analyzed at least every 8 hour shift in accordance with good laboratory practice. Refer to Westgard et al for identification and resolution for out of control situations.

## RESULTS

Factor X results are reported in activity (%).

## SPECIMEN COLLECTION

Nine parts of freshly drawn venous blood are collected into one part 3.2% trisodium citrate. Centrifugation: 2000 x g for 10 – 20 minutes at 20 - 25°C. Refer to NCCLS document H21-A3 for further instructions on specimen collection, handling, and storage.

## RECOMMENDED MEASURING RANGE/DETECTION LIMIT

The FX concentration is linear between 10% of normal (detection limit) and 130% of normal.

## CALIBRATION

A standard curve is required for each DiaPharma Factor X kit. Five standards must be included in each test run. For standardization, use commercially supplied hemostasis calibration plasma, or a pool of calibrated fresh frozen normal plasma obtained in the same manner as the plasma to be tested.

## Preparation of Standards

% FX	Normal Plasma, mL	Buffer, mL
100	100	-
75	75	25
50	50	50
25	25	75
10	10	90

## PROCEDURE

### Microplate Method

Predilution of samples: The predilution is performed to prevent interference from inhibitors, lipemic samples, icteric or hemolysed samples.

Standards, plasma samples, and controls 50 µl  
Buffer 1000 µl

## PROCEDURE – CONTINUED

### Kinetic Method

Step	Amount / Time
Add pre-diluted Sample	50 µl
Incubate at 37°C	3 – 4 min.
Add chromogenic substrate	50 µl
Mix well and add within	30 sec
RVV + CaCl <sub>2</sub> (20 - 25°C)	50 µl
Mix well	

Transfer the microplate to a microplate reader immediately after addition of the RVV + CaCl<sub>2</sub> and measure the absorbance change in a microplate reader at 405 nm. The microplate reader must be pre-incubated at 37°C.

If performing the acid-stopped method, mix and incubate at 37°C for 3 minutes after addition of RVV + CaCl<sub>2</sub>, then add 50 µl of 20% Acetic acid. Read the absorbance of the samples at 405 nm. The color is stable for at least 4 hours.

### DETERMINATIONS PER KIT

Microplate method: approximately 400

Automated methods: approximately 200 – will vary according to analyzer

### CALCULATION

Plot the change in absorbance per minute ( $\Delta A/\text{min}$ ) for the standard samples against their concentration of FX. Read the %FX value for the corresponding absorbance for the unknown sample from the standard curve.

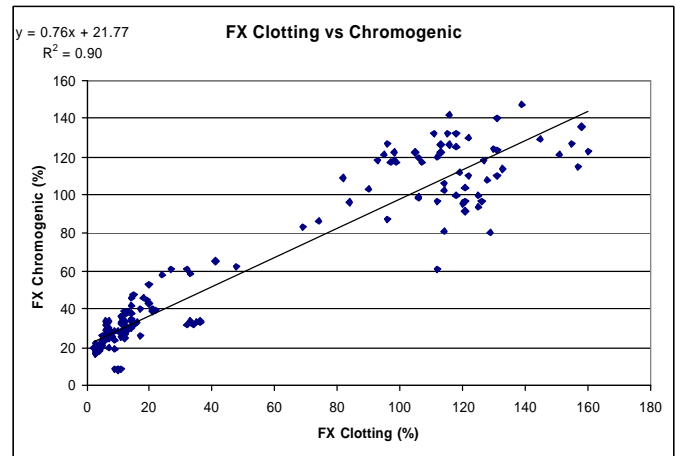
### PERFORMANCE CHARACTERISTICS

A linear regression of the FX clotting versus chromogenic assay was performed, yielding:  
FX Chromogenic = 0.76 · FX Clotting + 21.77 with a correlation of  $R^2 = 0.90$

Confidence Interval for Slope & Intercept:

FX Clotting (%)	FX Chromogenic (%)
Confidence Level (95.0%) 8.25	Confidence Level (95.0%) 6.65

	Lower 95.0%	Upper 95.0%
Intercept	18.77	24.78
X Variable	0.72	0.80



### PRECISION

Six samples were assayed in one FX kit run in replicates of 10 using a microplate. The samples were two controls (one normal and one prolonged), two patient samples with normal levels, and two patients with prolonged levels.

#### Precision Data

FX Concentration (%)	CV Within Run
Normal (98 – 107%)	1.9 – 12.0%
Abnormal (23 – 32%)	4.7 – 19.6%

The central tendency for within-run precision of the DiaPharma Factor X kit is approximately 7 %CV.

### ACCURACY

Clinical studies were done with a total of 241 individuals (92 normals + 149 OAC patients) across three sites. Citrated plasma samples from each individual were assayed by the FX Kit and by the routine prothrombin method at each site. The data were compared by log-log linear regression, and showed a correlation coefficient ("r") of 0.89.

### LIMITATIONS AND INTERFERING FACTORS

- Bilirubin, hemoglobin, and plasma from hyperlipemic patients give difficulties in absorbance readings. In these cases individual plasma blanks are necessary.
- Heparin concentrations below 30 U/ml are neutralized by Polybrene™ and do not influence the assay.
- Temperature control and incubation times are critical.
- Proper mixing is important to be sure that the reaction mixture is homogenous, but avoid vigorously shaking, as the proteins may precipitate in the foam.

### EXPECTED VALUES

Investigations have been made of concentrations in plasma obtained from:

#### 1) Normal subjects

DiaPharma Factor X has been evaluated in a study with 65 subjects. The samples were tested according to standard procedure, and the range of results (90% limits) was 130% to 59%. These limits corroborate data in the literature.

#### 2) Anticoagulant therapy patients

DiaPharma Factor X has been evaluation in a study of 149 patients undergoing oral anticoagulant. The range of Factor X concentrations in plasma of anticoagulant therapy patients was 13 to 79%, with a mean of 30%.

### REFERENCES

- 1) Sanfelippo, MJ, Sennet, J, McMahon, EJ, "Falsely Elevated INRs in Warfarin-Treated Patients with the Lupus Anticoagulant," Wisconsin Medical Journal, June, 2000; 62-64, 43.
- 2) Moll, S, Ortel, TL, "Monitoring Warfarin Therapy in Patients with Lupus Anticoagulants," Annals of Internal Medicine, Vol. 127, No. 3, 1 August 1997;177-185
- 3) Coatest® FX Package Insert. Chromogenix/Instrumentation Laboratory.
- 4) Lind SE, Callas PW, Golden EA, Joyner KA Jr, Ortel TI. Plasma Levels of Factors II, VII, and X and their Relationship to the International Normalized Ratio During Chronic Warfarin Therapy. Blood Coagul Fibrinolysis. 1997; 8: 48-53.

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