FOR RESEARCH USE ONLY!

Factor Xa Activity Fluorometric Assay Kit

07/14

(Catalog # K361-100; Store kit at -20°C)

I. Introduction:

Factor Xa (FXa) is the activated form of the coagulation factor X (Stuart-Power factor, thrombokinase, prothrombinase, thromboplastin, E.C.3.4.21.6). Factor X, a serine endopeptidase plays an important role at several stages of the coagulation pathway. It acts by converting prothrombin into active thrombin by complexing with activated co-factor V in the prothrombinase complex. Unfractionated heparin and various low molecular weight heparins bind to plasma cofactor antithrombin to inactivate several coagulation factors including factor Xa. Biovision's Factor Xa activity assay kit utilizes the ability of Factor Xa to cleave a synthetic substrate thereby releasing a fluorophore, AMC, which can be quantified by fluorescence readers. This assay kit is simple, rapid and can detect Factor Xa activity as low as 1 ng.

FXa Substrate-AMC $\xrightarrow{\text{FXa Enzyme}}$ Cleaved Substrate + AMC (Fluorescence)

II. Applications:

- Determine activity of pure Factor Xa
- Detect the activity of Factor Xa in plasma

III. Kit Contents:

Components	K361-100	Cap Code	Part Number
FXa Dilution Buffer	1 ml	Clear	K361-100-1
FXa Assay Buffer	15 ml	WM	K361-100-2
FXa Enzyme Standard	5 µl	Green	K361-100-3
FXa Substrate	0.2 ml	Red	K361-100-4

IV. User Supplied Reagents and Equipment:

- 96-well microplate with flat bottom. White plate is preferred for this assay.
- Multi-well spectrophotometer.

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials at low speed prior to opening. Read entire protocol before performing the assay.

- **FXa Assay Buffer:** Bring to room temperature before use.
- **FXa Enzyme Standard:** Prepare a stock solution of FXa Enzyme (100 ng/µl) by adding 45 µl of FXa Dilution buffer to 5 µl of FXa Enzyme Standard. Mix. Aliquot & store at -80°C. Avoid repeated freeze/thaw.

VI. Factor Xa Activity Assay Protocol:

1. **Sample Preparation:** Add 2-50 µl of sample containing FXa per well of 96-well plate and adjust the volume to 50 µl with FXa Assay Buffer.
2. **Standard Curve Preparation**: Dilute FXa Enzyme Standard to 5 ng/µl by adding 95 µl of FXa Dilution Buffer to 5 µl of FXa Enzyme stock solution (100 ng/µl). Mix and add 0, 4, 8, 12, 16 and 20 µl of diluted FXa Enzyme Standard (5 ng/µl) into a series of wells in a 96-well plate. Adjust the volume to 50 µl with FXa Assay Buffer to prepare 0, 20, 40, 60, 80 and 100 ng/well of FXa Enzyme Standard.

   For more sensitive assay, prepare Standard Curve of FXa ranging from 1-10 ng, dilute 5 ng/µl FXa Standard solution further to 0.5 ng/µl by adding 10 µl of 5 ng/µl FXa Standard solution to 90 µl of FXa dilution buffer. Mix and add 0, 4, 8, 12, 16, and 20 µl of 0.5 ng/µl FXa Enzyme Standard into a series of wells in a 96-well plate. Adjust the volume to 50 µl with FXa Assay Buffer to prepare 0, 2, 4, 6, 8 and 10 ng/well of FXa Enzyme Standard.

   Note: Diluted FXa Enzyme Standard solution is stable at 4°C for up to one week.
3. **Substrate Mix:** Prepare enough reagents for the number of assays to be performed. Prepare 50 µl of Substrate Mix for Standard & sample wells.

FXa Assay Buffer	48 µl
FXa Substrate	2 µl

   Mix and add 50 µl of FXa Substrate Mix into Standard and sample well(s). Mix well.
4. **Measurement:** Measure fluorescence in kinetic mode for 30-60 min. at 37°C (Ex/Em = 350/450 nm). Choose two time points (T_1 & T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU1 and RFU2). To reduce the background from sample, fluorescence can be read at Ex/Em = 350/460 nm or Ex/Em = 350/470 nm. However, the sensitivity may be lower at these wavelengths.
5. **Calculations**: Subtract 0 Standard reading from all readings. Plot the Factor Xa Standard Curve. Apply sample's ΔRFU to FXa Standard Curve to obtain corresponding FXa (B, in ng) and calculate the activity of FXa in the sample as:

$$\textbf{Sample FXa Activity} = \frac{\boldsymbol{B}}{\mathbf{V}} \times \boldsymbol{Dilution\ Factor} = \frac{\boldsymbol{ng}}{\boldsymbol{ml}} = \frac{\boldsymbol{\mu g}}{\boldsymbol{L}}$$

Where **B** is FXa amount from Standard Curve (ng)
V is the sample volume added into the reaction well (ml)

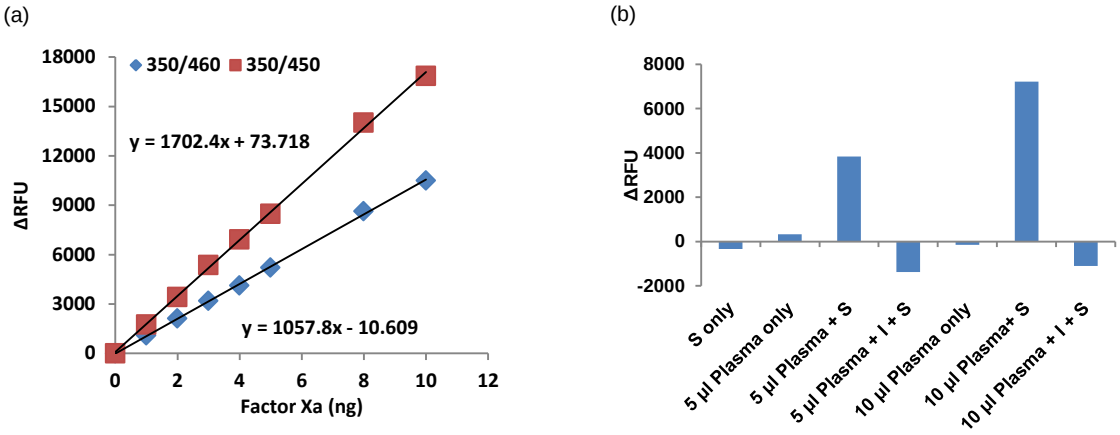

Figure: (a) Standard plot of FXa activity measured at two different emission wavelengths (450 and 460 nm) keeping the excitation at 350 nm. (b) FXa activity was measured in plasma samples in the presence and absence of a FXa inhibitor, GGACK Dihydrochloride. S = Substrate, I = Inhibitor. Assays were performed following the kit protocol.

VII. RELATED PRODUCTS:

Factor Va, Human Plasma (4098)
Thrombin, Active, Bovine Plasma (7592)
Antithrombin III (7298)
TFPI Antibody (3379)
Thrombin, Active, Human Plasma (7593)
Factor Xa Inhibitor Screening Kit (Fluorometric) (K362)

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