Factor Xa Inhibitor Screening Kit (Fluorometric)

(Catalog # BN00625; 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 inhibitor screening kit utilizes the ability of Factor Xa to cleave a synthetic substrate thereby releasing a fluorophore, AMC, which can be quantified by a fluorescence reader. In the presence of an inhibitor, the extent of the cleavage reaction is reduced or completely abolished. The loss in the fluorescence intensity can be correlated to the amount of inhibitor present in the assay solution. The Factor Xa Inhibitor Screening Kit is a simple, straightforward, high-throughput assay to screen Factor Xa inhibitors.

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

FXa Substrate-AMC $\xrightarrow{\text{FXa Enzyme + FXa inhibitor}}$ Decrease in fluorescence/No fluorescence

II. Applications:

- Screening potential inhibitors of Factor Xa
- Characterizing/studying Factor Xa inhibitors in plasma samples

III. Kit Contents:

Components	BN00625	Cap Code	Part Number
FXa Dilution Buffer	1 ml	Clear	BN00625-1
FXa Assay Buffer	15 ml	WM	BN00625-2
FXa Enzyme	5 µl	Green	BN00625-3
FXa Substrate	0.2 ml	Red	BN00625-4
FXa Inhibitor (GGACK Dihydrochloride, 10 mM)	10 µl	Blue	BN00625-5

IV. User Supplied Reagents and Equipment:

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

V. Storage Condition 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:** Add 105 µl of FXa Dilution Buffer to prepare stock solution. Mix. Aliquot & store at -80°C. Avoid repeated freeze/thaw.

VI. Factor Xa Inhibitor Screening Protocol:

1. **Enzyme Solution Preparation**: Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µl of FXa enzyme solution.

FXa Assay Buffer	49 µl
FXa Enzyme stock solution	1 µl

   Mix & add 50 µl of FXa Enzyme Solution into desired wells.

2. **Screening compounds, Inhibitor Control & Enzyme Control Preparations:** Dissolve candidate inhibitors into proper solvent. Dilute to 10X the desired test concentration with FXa Assay Buffer. Add 10 µl diluted test inhibitors (Sample, S) or FXa Assay Buffer into FXa Enzyme containing wells (Enzyme Control, EC). As an Inhibitor Control (IC), add 1 µl FXa Inhibitor and 9 µl FXa Assay Buffer to FXa Enzyme well(s). Incubate at room temperature for 10-15 min.

3. **Substrate Preparation:** For each well, prepare 40 µl of substrate solution.

FXa Assay Buffer	38 µl
FXa Substrate	2 µl

   Mix & add 40 µl of FXa Substrate solution into each well. 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). Irreversible inhibitors that inhibit the FXa activity completely at the tested concentration will have ΔRFU = 0 and will show 100% Relative Inhibition.

5. **Calculations**: Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU (RFU_2-RFU_1) values with the time ΔT (T_2-T_1).

$$\% \textbf{ Relative Inhibition} = \frac{\textbf{Slope of EC} - \textbf{Slope of S}}{\textbf{Slope of EC}} \times 100$$

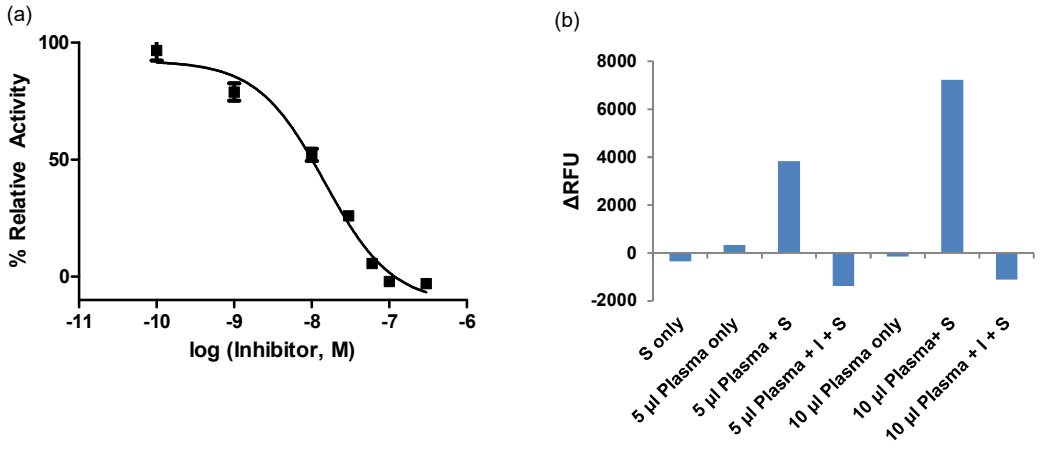

Figure: (a) Inhibition of FXa activity by FXa Inhibitor (GGACK Dihydrochloride). (b) FXa activity was measured in plasma samples in the presence and absence of FXa Inhibitor (GGACK Dihydrochloride). S = Substrate, I = Inhibitor. Assays were performed following the kit protocol.

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