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# Product Information

**Factor Xa Inhibitor Screening Kit (Fluorometric)**

Catalog Number **MAK239**
Storage Temperature –20 °C

## TECHNICAL BULLETIN

### Product Description

Factor Xa (FXa) is the activated form of the coagulation factor X (E.C.3.4.21.6, prothrombinase, Stuart-Power factor, thrombokinase, and thromboplastin,). 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.

This 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.

### Components

The kit is sufficient for 100 assays in 96 well plates.

| | |
|---|---|
| FXa Dilution Buffer<br>Catalog Number MAK239A | 1 mL |
| FXa Assay Buffer<br>Catalog Number MAK239B | 15 mL |
| FXa Enzyme<br>Catalog Number MAK239C | 5 µL |
| FXa Substrate<br>Catalog Number MAK239D | 0.2 mL |
| FXa Inhibitor (GGACK Dihydrochloride, 10 mM)<br>Catalog Number MAK239E | 10 µL |

### Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – white plates are preferred for this assay.
- Fluorescence multiwell plate reader

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Preparation Instructions

Briefly centrifuge small vials at low speed prior to opening.

FXa Assay Buffer – Bring to room temperature before use.

FXa Enzyme **–** Add 105 µL of FXa Dilution Buffer to prepare stock solution. Mix, aliquot, and store at –80 °C. Avoid repeated freeze/thaw cycles.

### Storage/Stability

Store the kit at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

### Procedure

Read entire protocol before performing the assay.

Enzyme Solution Preparation

Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of FXa enzyme solution, see Table 1.

**Table 1.**
Preparation of Enzyme Solution

| Reagents | Volume |
|---|---|
| FXa Assay Buffer | 49 µL |
| FXa Enzyme stock solution | 1 µL |

Mix and add 50 µL of FXa Enzyme Solution into desired wells.

Screening compounds, Inhibitor Control, and Enzyme Control Preparations

Dissolve candidate inhibitors to be tested into proper solvent. Dilute to 10× the desired test concentration with FXa Assay Buffer. Add 10 µL of diluted test inhibitors (Sample, S) or FXa Assay Buffer (Enzyme Control, EC) into FXa Enzyme containing wells. As an Inhibitor Control (IC), add 1 µL of FXa Inhibitor (Catalog Number MAK239E) and 9 µL of FXa Assay Buffer to FXa Enzyme well(s). Incubate at room temperature for 10–15 minutes.

Substrate Preparation

For each well, prepare 40 µL of substrate solution, see Table 2.

**Table 2.**
Preparation of Substrate

| Reagents | Volume |
|---|---|
| FXa Assay Buffer | 38 µL |
| FXa Substrate | 2 µL |

Mix and add 40 µL of FXa Substrate solution into each well. Mix well.

Measurement

Measure fluorescence in kinetic mode for 30–60 minutes at 37 °C ($\lambda_{ex}$ = 350 nm/$\lambda_{em}$ = 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 ($RFU_1$ and $RFU_2$). Irreversible inhibitors that inhibit the FXa activity completely at the tested concentration will have ΔRFU = 0 and will show 100% Relative Inhibition.

**Results**

Calculations

Calculate the slope for all Samples (S), including Enzyme Control (EC), by dividing the net ΔRFU ($RFU_2$–$RFU_1$) values by the time ΔT ($T_2$–$T_1$).

% Relative Inhibition = [Slope(EC)–Slope(S)]/Slope(EC) × 100

**Troubleshooting Guide**

| Problem | Possible Cause | Suggested Solution |
|---|---|---|
| Assay Not Working | Cold assay buffer | Assay Buffer must be at room temperature |
| | Omission of step in procedure | Refer and follow Technical Bulletin precisely |
| | Plate reader at incorrect wavelength | Check filter settings of instrument |
| | Type of 96 well plate used | White plates are preferred for this assay. |
| Samples with erratic readings | Samples prepared in different buffer | Use the Assay Buffer provided or refer to Technical Bulletin for instructions |
| | Cell/Tissue culture samples were incompletely homogenized | Repeat the sample homogenization, increasing the length and extent of homogenization step. |
| | Samples used after multiple freeze-thaw cycles | Aliquot and freeze samples if samples will be used multiple times |
| | Presence of interfering substance in the sample | If possible, dilute sample further |
| | Use of old or inappropriately stored samples | Use fresh samples and store correctly until use |
| Lower/higher readings in samples and standards | Improperly thawed components | Thaw all components completely and mix gently before use |
| | Use of expired kit or improperly stored reagents | Check the expiration date and store the components appropriately |
| | Allowing the reagents to sit for extended times on ice | Prepare fresh Reaction Mix before each use |
| | Incorrect incubation times or temperatures | Refer to Technical Bulletin and verify correct incubation times and temperatures |
| | Incorrect volumes used | Use calibrated pipettes and aliquot correctly |
| Non-linear standard curve | Use of partially thawed components | Thaw and resuspend all components before preparing the reaction mix |
| | Pipetting errors in preparation of standards | Avoid pipetting small volumes |
| | Pipetting errors in the Reaction Mix | Prepare a Reaction Mix whenever possible |
| | Air bubbles formed in well | Pipette gently against the wall of the plate well |
| | Standard stock is at incorrect concentration | Refer to the standard dilution instructions in the Technical Bulletin |
| | Calculation errors | Recheck calculations after referring to Technical Bulletin |
| | Substituting reagents from older kits/lots | Use fresh components from the same kit |
| Unanticipated results | Samples measured at incorrect wavelength | Check the equipment and filter settings |
| | Samples contain interfering substances | If possible, dilute sample further |
| | Sample readings above/below the linear range | Concentrate or dilute samples so readings are in the linear range |

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