Technical Bulletin

Factor Xa Activity Fluorometric Assay Kit

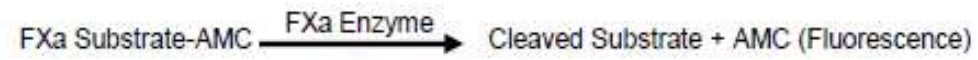
Catalog Number MAK238

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 is a serine endopeptidase which 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 Activity Assay Kit utilizes the ability of Factor Xa to cleave a synthetic substrate, releasing a fluorophore (AMC) which can be quantified by fluorescence readers. This assay kit is simple, rapid, and can detect activity from as low as 1 ng of Factor Xa.

The kit is suitable for determining the activity of pure Factor Xa and for detecting the activity of Factor Xa in plasma.



Components

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

- FXa Dilution Buffer — 1 mL
  Catalog Number MAK238A
- FXa Assay Buffer — 15 mL
  Catalog Number MAK238B
- FXa Enzyme Standard — 5 µL
  Catalog Number MAK238C
- FXa Substrate — 0.2 mL
  Catalog Number MAK238D

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (e.g., multichannel pipettor)
- Fluorescence multiwell plate reader
- White flat-bottom 96-well plates. Cell culture or tissue culture treated plates are **not** recommended.
- Microcentrifuge

Precautions and Disclaimer

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.

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Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C, protected from light.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

FXa Assay Buffer – Bring to room temperature prior to 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, and store at –80 °C. Avoid repeated freeze/thaw cycles.

Procedure

Sample Preparation

Add 2–50 µL of Sample containing FXa per well of a 96-well plate and adjust the total volume to 50 µL with FXa Assay Buffer.

Standard Curve Preparation (0-100 ng/well)

1. Prepare a 5 ng/µL Factor Xa Standard by mixing 5 µL of the 100 ng/µL FXa Enzyme Stock Solution with 95 µL of FXa Dilution Buffer.
2. Prepare Factor Xa Standards in separate wells of the 96-well plate according to Table 1.

   Note: The diluted FXa Enzyme Standard solution is stable at 4 °C for up to one week.

Table 1.
Preparation of 0-100 ng/well Factor Xa Standards

Well	5 ng/µL Factor Xa Standard	FXa Assay Buffer	Factor Xa (ng/well)
1	0 µL	50 µL	0
2	4 µL	46 µL	20
3	8 µL	42 µL	40
4	12 µL	38 µL	60
5	16 µL	34 µL	80
6	20 µL	30 µL	100

Standard Curve Preparation (0-10 ng/well)

For a more sensitive assay, prepare standards of FXa ranging from 1–10 ng.

1. Prepare a 5 ng/µL Factor Xa Standard by mixing 5 µL of the 100 ng/µL FXa Enzyme Stock Solution with 95 µL of FXa Dilution Buffer.
2. Further dilute the 5 ng/µL Factor Xa Standard to 0.5 ng/µL by adding 10 µL of the 5 ng/µL FXa Standard solution to 90 µL of FXa Dilution Buffer.
3. Prepare Factor Xa Standards in separate wells of the 96-well plate according to Table 2.

   Note: The diluted FXa Enzyme Standard solution is stable at 4 °C for up to one week.

Table 2.
Preparation of 0-10 ng/well Factor Xa Standards

Well	0.5 ng/µL Factor Xa Standard	FXa Assay Buffer	Factor Xa (ng/well)
1	0 µL	50 µL	0
2	4 µL	46 µL	2
3	8 µL	42 µL	4
4	12 µL	38 µL	6
5	16 µL	34 µL	8
6	20 µL	30 µL	10

Master Reaction Mix

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Master Reaction Mix according to Table 3. Mix well.

Table 3.
Preparation of Master Reaction Mix

Reagent	Master Reaction Mix
FXa Assay Buffer	48 µL
FXa Substrate	2 µL

2. Add 50 µL of Master Reaction Mix into each Standard and Sample well. Mix well.

Measurement

1. Measure fluorescence in kinetic mode for 30-60 minutes at 37 °C (λ_{Ex} = 350 nm/ λ_{Em} = 450 nm).
2. Choose two time points (T_1 and T_2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFU_1 and RFU_2).

   Note: To reduce the background from Sample, fluorescence can be read at λ_{Ex} = 350 nm/λ_{Em} = 460 nm or λ_{Ex} = 350 nm/λ_{Em} = 470 nm. However, the sensitivity may be lower at these emission wavelengths.

Results

1. Calculate ΔRFU (RFU_2 – RFU_1) values for all Samples and Standards.
2. Subtract the 0 Standard ΔRFU value from all ΔRFU values.
3. Plot the Factor Xa Standard Curve.
4. Apply the ΔRFU of the Sample to the FXa Standard Curve to obtain the corresponding FXa (B, in ng).
5. Calculate the activity of Factor Xa in the Sample:

   Factor Xa Activity (ng/mL or µg/L) =

$$\frac{B}{V} \; x \; DF$$

where

B = FXa amount from Standard Curve (ng)

V = Sample volume added into the reaction well (mL)

DF = Sample dilution factor (DF = 1 for undiluted Samples)

Figure 1.
Typical standard curve of Factor Xa activity measured at two different emission (λ_{Em}) wavelengths (450 and 460 nm), keeping the excitation wavelength (λ_{Ex}) at 350 nm.

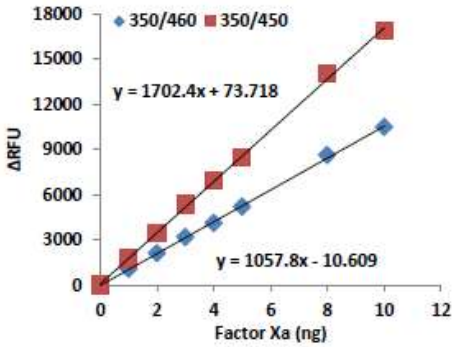
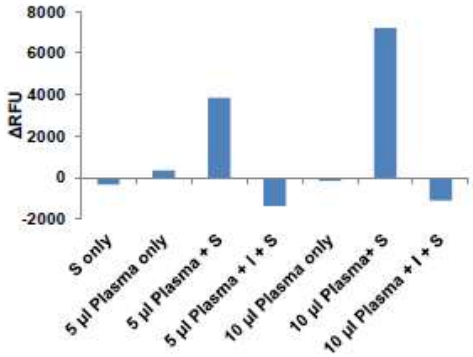


Figure 2.
Factor Xa activity was measured in plasma samples in the presence and absence of a Factor Xa inhibitor, GGACK Dihydrochloride. S = Substrate, I = Inhibitor. Assays were performed following the kit protocol.



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