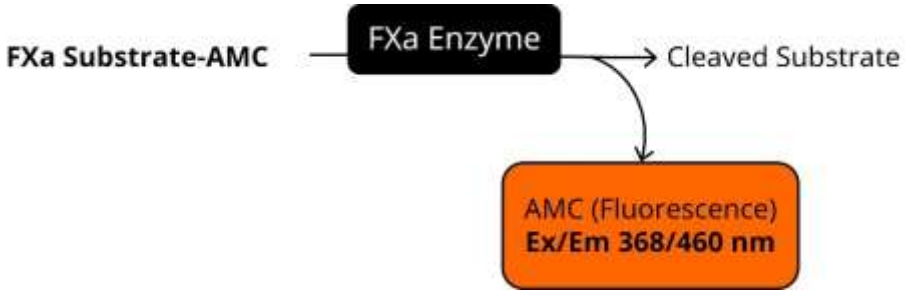
ab325358 - Factor Xa Activity Assay Kit (Fluorometric)

For rapid, sensitive and accurate measurement of Factor Xa activity in various samples.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325358

Background:

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 o plasma cofactor antithrombin to inactivate several coagulation factors including factor Xa. Abcam's Factor Xa Activity Assay Kit utilizes the ability of Factor Xa to cleave a synthetic peptide substrate thereby releasing a fluorophore, AMC, which can be quantified by fluorescence readers. This assay is simple, rapid, and can detect Factor Xa activity as low as 1 ng, or 35 µU/well.



Assay Summary:

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

Sample preparation

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Standard curve preparation

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Add reaction mix to samples and incubate 30-60 min at 37°C

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Measure fluorescence (Ex/Em = 368/460 nm) on a microplate reader

QUICK ASSAY PROCEDURE

- Thaw kit components and aliquot if necessary; get equipment ready.
- Prepare standard curve and samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (100 µL), samples (50 µL) and background controls (50 µL).
- Prepare FXa Substrate Mix and add 50 µL FXa Substrate Mix to each sample/BG well.
- Measure fluorescence at Ex/Em= 368/460 nm for 30-60 minutes at 37°C.

Precautions & Limitations:

Please read these instructions carefully prior to beginning the assay.
All kit components have been formulated and quality control tested to function successfully as a kit.

- Modifications to the kit components or procedures may result in loss of performance.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

Storage and Stability:

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
FXa Dilution Buffer	1 mL	-20°C	-20°C
FXa Assay Buffer	15 mL	-20°C	-20°C
AMC Standard (1 mM AMC in DMSO)	100 µL	-20°C	-20°C
FXa Substrate (200 nmol)	200 µL	-20°C	-20°C
FXa Enzyme	5 µL	-20°C	-80°C

Materials Required, Not Supplied:

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring fluorescence at Ex/Em = 368/460 nm.
- 96 well black plate with flat bottom.

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

FXa Assay Buffer: Bring to room temperature prior to use.

FXa Dilution Buffer and FXa Substrate are ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C and protect from light and moisture.

FXa Enzyme: Dilute FXa Enzyme to 100 ng/µL by adding 45 µL of FXa Dilution Buffer to 5 µL of FXa Enzyme. Aliquot and store excess at -80°C. Further dilute 100 ng/µL FXa Enzyme to a 5 ng/µL working solution by adding 5 µL of 100 ng/µL solution with 95 µL of Dilution Buffer. Do not store 5 ng/µL FXa Enzyme solution after use.

AMC Standard: Dilute stock AMC Standard to 100 µM working solution by adding 10 µL AMC stock to 90 µL ddH_2O. Do not store diluted AMC working solution after use.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

Prepare serial dilution of AMC Standard as follows:

Use 100 µM AMC Standard working solution as follows to generate 0, 200, 400, 600, 800, and 1000 pmole/well standard curve:

Standard #	Volume of 100 µM AMC Standard (µL)	FXa Assay Buffer (µL)	Final volume standard in well (µL)	AMC Concentration (pmoles/well)
1	0	100	100	0
2	2	98	100	200
3	4	96	100	400
4	6	94	100	600
5	8	92	100	800
6	10	90	100	1000

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples, and the readings can be lower than expected.

Plasma:

Plasma samples can be tested directly by adding samples to microplate wells.

Purified Protein Samples:

Dilute purified protein in FXa Assay Buffer before adding directly to microplate wells.

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay. We recommend that you assay all standards, controls, and samples in duplicate.
- Equilibrate all other materials and prepare reagents to room temperature prior to use.

1. Set up Reaction wells:
   - Standard wells = 100 µL AMC standard dilutions.
   - Positive Control wells = 2 µL of 5 ng/µL FXa Enzyme solution.
   - Sample wells = 2 – 50 µL samples (adjust volume to 50 µL/well with FXa Assay Buffer).
   - Substrate-Only Background Control wells = 50 µL FXa Assay Buffer.
2. Each sample and substrate-only background control well requires 50 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a bulk mix of the Reaction Mix.

Component	Reaction Mix (µL)
FXa Assay Buffer	48
FXa Substrate	2

3. Mix bulk Reaction Mix by inversion. Add 50 µL of the bulk Reaction Mix to each sample and background control well. Use a clean tip for each well.
4. Measure fluorescence immediately on a microplate reader at Ex/Em= 368/460 nm in kinetic mode for 30-60 minutes at 37°C.

Note: If measuring endpoint fluorescence, incubate plate 30-60 minutes at 37°C, then measure fluorescence on a microplate reader at Ex/Em = 368/460 nm in endpoint mode.

Calculations:

- For samples producing signals greater than those of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.

1. Average duplicate reading for each standard and sample.
2. Subtract the mean fluorescence value (RFU) of the blank (Standard #1) from all standard readings. This is the corrected RFU.
3. Plot the corrected RFU values for each standard as a function of the amount of AMC in the well (in pmoles) and determine the equation of the standard curve using a linear regression.
4. For all reaction wells (including substrate-only background control wells), choose two time points (T_1 and T_2) in the linear phase of the reaction progress curves and obtain the corresponding RFU values at those points (RFU_1 and RFU_2).
5. Calculate ΔRFU for samples as follows:

   $\Delta RFU_{368/460} = RFU_2 - RFU_1$

   Note: As assay is run at 37°C, it may take a few minutes for sample to reach the required temperature, creating an initial "lag phase." Choose progress curve time points accordingly.
6. Determine the background-corrected change in fluorescence intensity for each well of sample by subtracting the ΔRFU value of the substrate-only background control (BC) from the ΔRFU value of the sample.
7. Using the slope of the AMC Standard Curve and the background-corrected sample ΔRFU values ($\Delta RFU_{Sample} - \Delta RFU_{BC}$), obtain *B* nmol of AMC generated by the sample during the reaction time:

   $B = \left(\frac{\Delta RFU_{Sample} - \Delta RFU_{BC}}{Slope}\right)$ = pmoles of substrate cleaved ≡ pmoles AMC

8. Calculate activity of Factor Xa in sample using the following equation:

$$FXa\ Activity = \left(\frac{B}{V \times \Delta T}\right) \times D = \frac{\text{pmoles}}{\text{min}} \times \text{mL} = \ \mu\text{U/mL}$$

Where:
B = amount of FXa from standard curve (in pmoles)
ΔT = reaction time (in minutes)
V = amount of sample volume added in sample wells (in mL)
D = sample dilution factor (D = 1 for undiluted samples)

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

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