

ab204711

Factor Xa Activity Assay Kit (Fluorometric)

Instructions for Use

For rapid, sensitive and accurate detection of Factor Xa activity.

This product is for research use only and is not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

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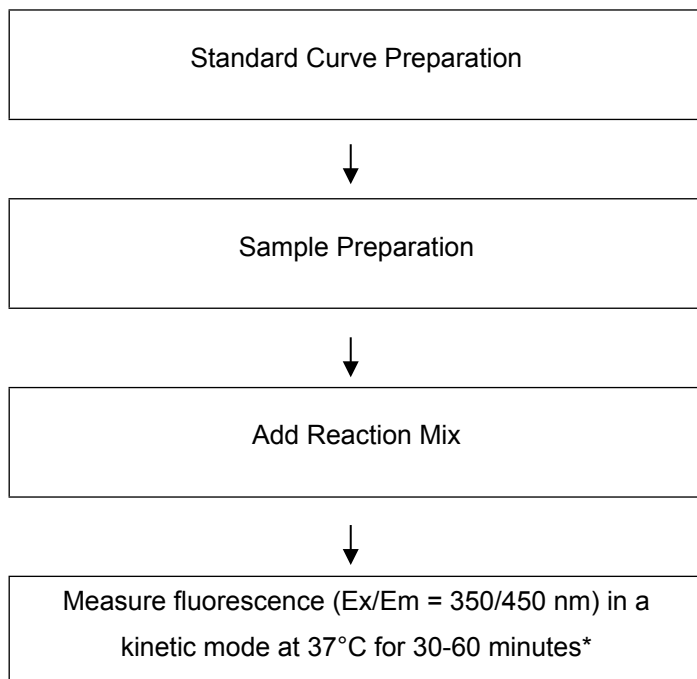
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1. BACKGROUND

Factor Xa Activity Assay Kit (Fluorometric) (ab204711) 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.

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.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

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.

4. 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.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- 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.

6. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
FXa Dilution Buffer	1 mL	-20°C	-20°C
FXa Assay Buffer	15 mL	-20°C	-20°C
FXa Enzyme/FXa Enzyme Standard	5 µL	-20°C	-80°C
FXa Substrate	200 µL	-20°C	-20°C

7. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microcentrifuge
- Pipettes and pipette tips
- Fluorescent microplate reader – equipped with filter Ex/Em = 350/450 nm
- 96 well plate with clear flat bottom preferably white
- Heat block or water bath

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Keep enzymes, heat labile components and samples on ice during the assay.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 **FXa Assay Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 **FXa Dilution Buffer:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.3 **FXa Enzyme/FXa Enzyme Standard:**

Prepare a stock solution of FXa Enzyme (100 ng/μL) by adding 45 μL of FXa Dilution buffer to the 5 μL of FXa Enzyme/FXa Enzyme Standard provided in the vial. Mix well by pipetting up and down. Aliquot enzyme so that you have enough volume to perform the desired number of assays. Avoid repeated freeze/thaw. Store at -80°C. Keep on ice while in use.

9.4 **FXa Substrate:**

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is can be stored at 4°C. Use within one week.

10.1 Prepare a 0.5 ng/μL FXa Enzyme/FXa Enzyme Standard by diluting 20 μL of the 5 ng/μL FXa Enzyme/FXa Enzyme Standard (Step 10.1) with 180 μL of FXa Dilution Buffer.

10.2 Using 0.5 ng/μL FXa Enzyme/FXa Enzyme Standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes.

Standard #	Volume of Standard (μL)	Assay Buffer (μL)	Final volume standard in well (μL)	End Conc FXa Enzyme in well (ng/well)
1	0	150	50	0
2	12	138	50	2
3	24	126	50	4
4	36	114	50	6
5	48	102	50	8
6	60	90	50	10

Each dilution has enough amount of standard to set up duplicate readings (2 x 50 μL).

11. SAMPLE PREPARATION

General Sample information:

- 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, if that is not possible, we suggest that you snap your samples in liquid nitrogen upon extraction and store the samples 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.

11.1 **Plasma:**

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

11.2 **Purified protein:**

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

NOTE: *To find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.*

12. ASSAY PROCEDURE and DETECTION

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- It is recommended to assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Set up Reaction wells:

- Standard wells = 50 μ L standard dilutions.
- Sample wells = 2 – 50 μ L samples (adjust volume to 50 μ L/well with FXa Assay Buffer).

12.2 Reaction Mix:

Prepare 50 μ L of Reaction Mix for each reaction:

Component	Reaction Mix (μ L)
FXa Assay Buffer	48
FXa Substrate	2

Mix enough reagents for the number of assays (samples and controls) to be performed. Prepare a master mix of the Reaction Mix to ensure consistency. We recommend the following calculation: X μ L component x (Number reactions +1)

- 12.3 Add 50 μ L of Reaction Mix into each standard and sample well. Mix well.
- 12.4 Measure output on a fluorescent microplate reader at Ex/Em = 350/450 nm in a kinetic mode, every 2 – 3 minutes, for 30-60 minutes at 37°C protected from light.

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.

NOTE: Sample incubation time can vary depending on Factor Xa (FXa) activity in the samples. We recommend measuring fluorescence in kinetic mode and then choosing two time points (T_1 and T_2) during the linear range.

RFU value at T_2 should not exceed the highest RFU in the standard curve.

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of FXa Enzyme.

13.4 Draw the best smooth curve through these points to construct the standard curve. Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Use the $\Delta\text{RFU}_{350/450\text{nm}}$ to obtain B (in ng) of Factor Xa.

13.6 Activity of Factor Xa in the test is calculated as:

$$\text{FXa Activity} = \left(\frac{B}{V}\right) * \text{Dilution Factor} = \text{ng/mL} = \mu\text{g/L}$$

Where:

B = Amount of FXa from Standard Curve (ng).

V = Original sample volume added into the reaction well (mL).

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

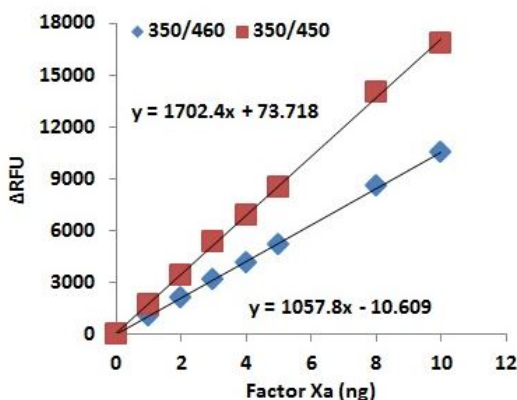


Figure 1. Standard plot of FXa activity measured at two different emission wavelengths (450 and 460 nm) keeping the excitation at 350 nm.

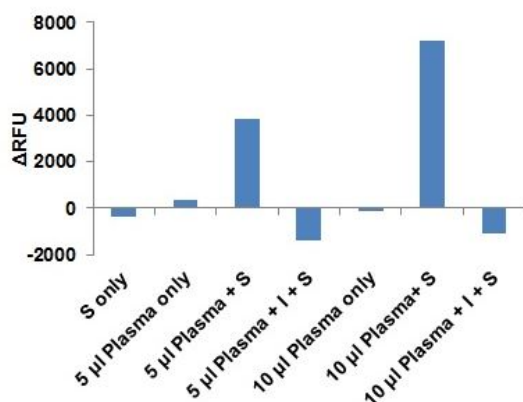


Figure 2. FXa activity was measured in plasma samples in the presence and absence of a FXa inhibitor, GGACK Dihydrochloride. S = Substrate, I = Inhibitor.

15. QUICK ASSAY PROCEDURE

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

- Prepare standard and prepare enzyme mix; get equipment ready.
- Prepare appropriate standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 μ L) and samples (50 μ L) wells.
- Prepare Factor Xa Reaction Mix (Number samples + standards + 1).

Component	Reaction Mix (μ L)
FXa Assay Buffer	48
FXa Substrate	2

- Add 50 μ L of Factor Xa Reaction Mix to the standard and sample wells.
- Incubate plate at 37°C during 30-60 minutes and read fluorescence at Ex/Em= 350/450 nm or or Ex/Em = 350/470 nm in a kinetic mode.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Clear plates
Sample with erratic readings	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μ L) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions on protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17.INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- **RIPA buffer** – it contains SDS which can destroy/decrease the activity of the enzyme.

18. FAQ

Technical Support

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