Version 6c Last updated 11 March 2026

ab154128
Factor Xa Assay Kit

For the measurement of Factor Xa activity in human plasma, serum and cell culture supernatant samples.

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

Table of Contents

1. Overview

Factor Xa Assay Kit (ab154128) is developed to determine Factor Xa activity in human plasma, serum and cell culture supernatant samples. The assay couples immunofunctional and direct amidolytic function. A polyclonal antibody specific for human Factor Xa has been pre-coated onto a microplate and Factor Xa is bound to the immobilized antibody. The amidolytic activity of the Factor Xa is quantitated by using a highly specific Factor Xa substrate releasing a yellow para-nitroaniline (pNA) chromophore. The change in absorbance of the pNA at 405 nm is directly proportional to the Factor Xa enzymatic activity.

Prepare all reagents, samples and standards as instructed.

Add 100 µL of standard or sample to each well. Cover and incubate overnight at 2-8 °C.

Wash and add 120 µL of 1X Diluent M and 20 µL of Factor Xa Substrate per well.

Read absorbance at 405 nm at zero minutes for background OD. Cover and incubate at 37°C.

Read absorbance at 405 nm at 22 hours. Cover and incubate at 37°C after each reading. Continue reading every 1 hour up to 28 hours.

2. Materials Supplied and Storage

Store kit at 4°C immediately upon receipt, apart from the Standard and FXa Substrate, which should be stored at -20°C.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in sections 5 & 6.

Item	Quantity	Storage temperature (before prep)
Human FXa Microplate	1 unit	+4°C
Sealing Tapes	3 units	+4°C
Diluent M Concentrate (10X)	20 mL	+4°C
Human FXa Standard	1 vial	-20°C
Wash Buffer Concentrate (20X)	30 mL	+4°C
FXa Substrate	2 vials	-20°C

3. Materials Required, Not Supplied

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

- Microplate reader capable of measuring absorbance at 405 nm.
- Deionized or distilled reagent grade water.
- Incubator (37°C).

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Prepare all reagents as instructed prior to running the assay. Freshly dilute all reagents and bring all reagents to room temperature before use. When diluting concentrates, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Mix the 1x solution gently until the crystals have completely dissolved.

5.1 Diluent M Concentrate (10X)

1. Dilute the Diluent M Concentrate 10-fold with reagent grade water to produce a 1X solution.

5.2 Wash Buffer Concentrate (20X)

1. Dilute the Wash Buffer Concentrate 20-fold with reagent grade water to produce a 1X solution.

5.3 FXa Substrate

Δ Note: Do not prepare FXa substrate until user is ready to continue to the chromogenic activity process.

1. Add 1.1 mL of reagent grade water to produce a 1X stock solution.
2. Allow the vial to sit for 10 minutes with gentle agitation prior to use; keep the vial on ice.
3. Aliquot remaining stock solution to limit repeated freeze-thaw cycles. The solution should be stored at -20°C and used within 10 days.

6. Standard Preparation

- **Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.**
- **Any remaining standard should be stored at -20°C after reconstitution and used within 30 days.**
- **This procedure prepares sufficient standard dilutions for duplicate wells.**

10.1 Reconstitution of the Human Factor Xa Standard vial to prepare the 8 mIU/mL (36 ng/ml) Human Factor Xa **Stock Standard**:

10.1.1 First consult the Human Factor Xa Standard vial to determine the mass of protein in the vial.

10.1.2 Calculate the appropriate volume of 1X Diluent M to add when resuspending the Human Factor Xa Standard vial to produce a 36 ng/mL Human Factor Xa **Stock Standard** by using the following equation:

C_S = Starting mass of Human Factor Xa Standard (see vial label) (ng)

C_F = 36 ng/mL Human Factor Xa **Stock Standard** final required concentration

V_D = Required volume of 1X Diluent M for reconstitution (µL)

Calculate total required volume 1X Diluent M for resuspension:

$$(C_S / C_F) \times 1{,}000 = V_D$$

Example:

NOTE: This example is for demonstration purposes only. Please remember to check your standard vial for the actual amount of standard provided.

C_S = 18 ng of Human Factor Xa Standard in vial

C_F = 36 ng/mL Human Factor Xa **Stock Standard** final concentration

V_D = Required volume of 1X Diluent M for reconstitution

(18 ng / 36 ng/mL) x 1,000 = 500 µL

10.1.3 First briefly spin the Human Factor Xa Standard Vial to collect the contents on the bottom of the tube.

10.1.4 Reconstitute the Human Factor Xa Standard vial by adding the appropriate calculated amount V_D of 1X Diluent M to the vial to generate the 36 ng/mL Human Factor Xa **Stock Standard**. Mix gently and thoroughly.

10.2 Allow the reconstituted 36 ng/mL Human Factor Xa **Stock Standard** to sit for 10 minutes with gentle agitation prior to making subsequent dilutions

10.3 Label eight tubes #1 – 8

10.4 Prepare the 9 ng/mL **Standard #1** by adding 250 µL of the reconstituted 36 ng/mL Human Factor Xa **Stock Standard** to 750 µL of 1X Diluent M and mix thoroughly and gently

10.5 Add 120 µL of 1X Diluent M to tubes #1 – 8.

10.6 To prepare **Standard #2**, add 120 µL of the **Standard #1** into tube #2 and mix gently.

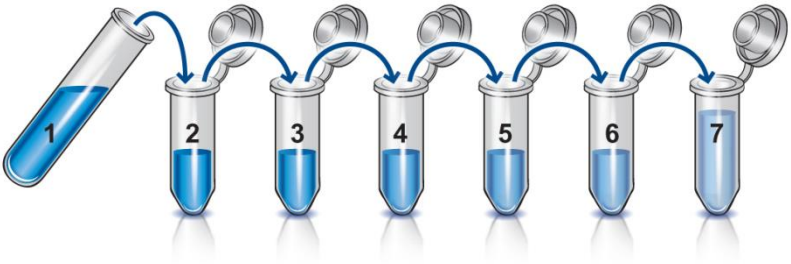
10.7 To prepare **Standard #3**, add 120 µL of the **Standard #2** into tube #3 and mix gently.

10.8 Using the table below as a guide, prepare subsequent serial dilutions.

10.9 1X Diluent M serves as the zero standard, 0 ng/mL (tube #8).

Standard Dilution Preparation Table

Standard #	Volume to Dilute (µL)	Volume Diluent M (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (mIU/mL)	Final Conc. (ng/mL)
1	Step 10.4				2.0	9.0
2	120	120	240	9.0	1.0	4.5
3	120	120	240	4.5	0.5	2.25
4	120	120	240	2.25	0.25	1.125
5	120	120	240	1.125	0.125	0.563
6	120	120	240	0.563	0.063	0.281
7	120	120	240	0.281	0.031	0.141
8	0	120	120	0	0	0



7. Sample Preparation

General sample information:

We recommend that you use fresh samples for the most reproducible assay. Avoid repeated freeze-thaw cycles.

7.1 Plasma

1. Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant.
2. Centrifuge samples at 3000 x *g* for 10 minutes and collect plasma.
3. The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs.
4. The undiluted samples can be stored at -20°C or below for up to 3 months

   ∆ **Note:** EDTA or Heparin can also be used as an anticoagulant.

7.2 Serum

1. Samples should be collected into a serum separator tube.
2. After clot formation, centrifuge samples at 3000 x *g* for 10 minutes and remove serum.
3. The sample is suggested for use at 1X; however, user should determine optimal dilution factor depending on application needs.
4. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

7.3 Cell Culture Supernatants

1. Centrifuge cell culture media at 3000 x *g* for 10 minutes at 4°C to remove debris and collect supernatants.
2. Samples can be stored at -20°C or below. Avoid repeated freeze-thaw cycles.

8. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

1. Prepare all reagents, standard solutions and samples for initial setup as instructed. Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccants inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator.
2. The assay is performed at 2-8°C for binding of standard and samples and at 37°C for chromogenic activity.
3. Add 100 µL of Human FXa Standard or sample to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate at 2-8°C overnight or for at least 12 hours.
4. Prepare FXa Substrate prior to washing the microplate. Allow the vial to sit for 10 minutes with gentle agitation prior to use.
5. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a machine, wash six times with 300 µL of Wash Buffer and then invert the plate, decanting the contents; hit 4-5 times on absorbent material to completely remove the liquid.
6. Add 120 µL of Diluent M (1X) to each well and add 20 µL of FXa Substrate to each well. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Read the absorbance at 405 nm for a zero minute background reading. Cover the wells with a sealing tape and incubate at 37°C in a humid incubator to avoid evaporation.
7. Read the absorbance (405 nm) at 22 hours and continue reading every hour up to 28 hours. Incubate microplate at 37°C after each reading.

9. Data Analysis

Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a Standard Curve from the optimal reaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute (ΔA/min) on the y-axis after subtracting the background. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

10. Typical Data

Data provided **for demonstration purposes only**.

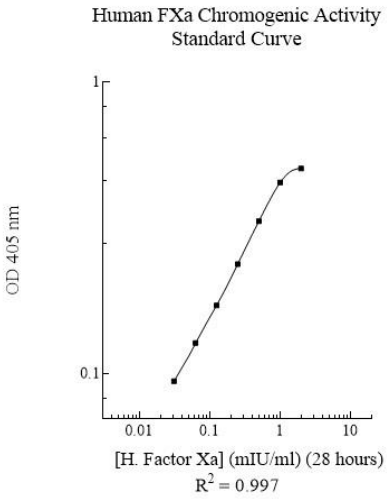


Figure 1. Human Factor Xa Chromogenic activity (ml/mU) Standard Curve.

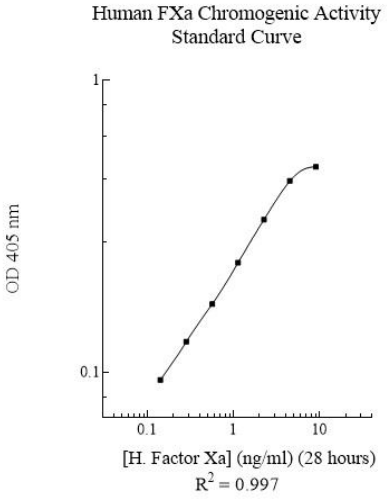


Figure 2. Human Factor Xa Chromogenic activity (ng/mL) Standard Curve.

SENSITIVITY

The minimum detectable dose of human FXa is approximately 0.022 mIU/mL.

This assay recognizes both natural and recombinant human FXa.

Δ **Note:** The conversion of mIU and ng is 21.85 mIU/mL = 100 ng/mL.

11. Troubleshooting

Problem	Cause	Solution
Low precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots.
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique.
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner.
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing.

Problem	Cause	Solution
Unexpectedly low or high signal intensity	Microplate was left unattended between steps.	Each step of the procedure should be performed uninterrupted.
	Omission of step	Consult the provided procedure for complete list of steps.
	Steps performed in incorrect order	Consult the provided procedure for the correct order.
	Insufficient amount of reagents added to wells	Check pipette calibration. Check pipette for proper performance.
	Wash step was skipped	Consult the provided procedure for all wash steps.
	Improper wash buffer	Check that the correct wash buffer is being used.
	Improper reagent preparation	Consult reagent preparation section for the correct dilutions of all reagents.
	Insufficient or prolonged incubation periods	Consult the provided procedure for correct incubation time.

Problem	Cause	Solution
Deficient standard curve fit	Non-optimal sample dilution	User should determine the optimal dilution factor for samples.
	Contamination of reagents	A new tip must be used for each addition of different samples or reagents during the assay procedure.
	Contents of wells evaporate	Verify that the sealing film is firmly in place before placing the assay in the incubator or at room temperature.
	Improper pipetting	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance.
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions.

12. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)