ab108833

Factor X Human Chromogenic Activity Assay Kit

Instructions for Use

For the quantitative measurement of Human Factor X activity in cell culture supernatants, serum, urine and plasma

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

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Table of Contents

1.	Introduction	2
2.	Assay Summary	3
3.	Kit Contents	4
4.	Storage and Handling	4
5.	Additional Materials Required	5
6.	Limitations	5
7.	Technical Hints	6
8.	Preparation of Reagents	7
9.	Assay Method	11
10.	Data Analysis	13
11.	Performance Characteristics	14

1. Introduction

Factor X is a plasma serine protease zymogen involved in the blood coagulation cascade. Factor X is purified from plasma as a two-chain protein consisting of a 45-kDa heavy chain and a 17-kDa light chain. The Factor X heavy chain is cleaved during coagulation by several different proteases, including the intrinsic Xase complex, the Factor X-activating enzyme from Russell's viper venom (RVV) and trypsin, and also by extrinsic (tissue Factor/Factor VIIa) pathway to give an active enzyme Factor Xa. Factor Xa as the activator of prothrombin occupies a central position linking the two blood coagulation pathways.

ab108833 is developed to determine Human Factor X activity in plasma, serum, urine and cell culture supernatants. The assay measures the activation of zymogen Factor X to Factor Xa by RVV. The amidolytic activity of the Factor Xa is quantitated 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 X enzymatic activity.

2. Assay Summary

Prepare all reagents, samples and standards as instructed.

Add 20 µl standard or sample.

Add 40 µl freshly prepared Assay Mix.

Read the absorbance at 405 nm at zero minutes for background O.D. Seal the plate with sealing tape and incubate at 37°C.

For high level of Factor X activity, read the absorbance at 405 nm every 5 minutes for 20 minutes.

For low level of Factor X activity, read the absorbance at 405 nm every 5 minutes for 80 minutes.

Cover and incubate at 37°C after each reading

3. Kit Contents

- Microplate: A 96-well polystyrene microplate (12 strips of 8 wells)
- Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes that can be cut to fit the format of the individual assay.
- Sample Diluent 6X: a six-fold concentrate (5ml)
- Human Factor X Standard: 1 vial
- Assay Diluent: A working solution (5ml)
- RVV: 1 vial
- Factor Xa Substrate: 2 vials

4. Storage and Handling

Store components of the kit at 2-8°C or -20°C upon arrival up to the expiration date. Store Factor X Standard, RVV, and FXa Substrate at -20°C. Store Microplate, Assay Diluent, and Sample Diluent at 2-8°C. Opened unused microplate wells may be returned to the foil pouch with the desiccant packs. Reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator. Diluent (1x) may be stored for up to 1 month at 2-8°C.

5. Additional Materials Required

- Microplate reader capable of measuring absorbance at 405nm.
- Precision pipettes to deliver 1 µl to 200 µl and multiple channel.
- Distilled or deionized reagent grade water.
- Incubator (37^{o}C).

6. Limitations

- 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.

7. Technical Hints

- It is recommended that all standards, controls and samples be run in duplicate. Standards and samples must be assayed at the same time.
- The coefficient of determination of the standard curve should be ≥ 0.95.
- Cover or cap all kit components and store at 2-8° C when not in use.
- Samples should be collected in pyrogen/endotoxin-free tubes.
- When possible, avoid use of badly hemolyzed or lipemic serum. If large amounts of particulate matter are present, centrifuge or filter prior to analysis.
- When pipetting reagents, maintain a consistent order of addition from well-to-well. This ensures equal incubation times for all wells.
- Do not mix or interchange different reagent lots from various kit lots.

8. Preparation of Reagents

Sample Collection:

1. **Plasma:** Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x *g* for 10 minutes and assay. Recommending diluting samples 1:20 with Sample Diluent, however, user should determine optimal dilution factor depending on application needs, and assay immediately. Samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA can also be used as an anticoagulant. Heparin is not recommended.)
2. **Serum:** Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum. Recommending diluting sample 1:20 with Sample Diluent, however, user should determine optimal dilution factor depending on application needs, and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
3. **Cell Culture Supernatants:** Centrifuge cell culture media at 1500 rpm for 10 minutes at 4°C to remove debris. Collect supernatants and assay. Samples can be stored at -80°C. Avoid repeated freeze-thaw cycles.
4. **Urine:** Collect urine using sample pot. Centrifuge samples at 800 x *g* for 10 minutes. Dilute samples 1:2, or between 1x

and 10x, into Sample Diluent; user should determine optimal dilution factor depending on application needs. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.

Reagent Preparation:

1. **Sample Diluent:** Dilute Sample Diluent Concentrate 1:6 with reagent grade water. When diluting the concentrate, 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.
2. **Standard Curve:** Reconstitute the Factor X Standard with the appropriate amount of Sample Diluent to generate a stock solution of 8 µg/ml. Allow the standard to sit for 10 minutes with gentle agitation prior to making dilutions.
3. For high level of Factor X standard, prepare duplicate or triplicate standard points by serially diluting the standard solution with equal volume of Sample Diluent to produce 4, 2, 1, 0.5, 0.25 and 0.125 µg/ml solutions. Sample Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20˚C.
4. For low level of Factor X standard, dilute stock solution (8 µg/ml) 1:8 with Sample Diluent to produce 1 µg/ml standard. Prepare duplicate or triplicate standard points by serially diluting the standard solution 1:4 with equal volume of Sample Diluent to produce 0.25, 0.0625 and 0.0156 µg/ml solutions. Sample Diluent serves as the zero standard (0 µg/ml). Any remaining solution should be frozen at -20˚C or below and used within 30 days.

Standard curve for high level of Factor X activity samples:

Standard Point	Dilution	[Factor X] (µg/ml)
P1	1 Part Standard (8 µg/ml) + 1 part Diluent	4.000
P2	1 part P1 + 1 part Diluent	2.000
P3	1 part P2 + 1 part Diluent	1.000
P4	1 part P3 + 1 part Diluent	0.500
P5	1 part P4 + 1 part Diluent	0.250
P6	1 part P5 + 1 part Diluent	0.125
P7	1 part P6 + 1 part Diluent	0.063
P8	Diluent	0.000

Standard curve for low level of Factor X activity samples:

5. RVV: Add 1.1 ml of Sample Diluent. Allow the RVV to sit for 10 minutes to dissolve. Any remaining solution should be frozen at -20°C or below and used within 30 days. Avoid repeated freeze-thaw cycles.

Standard Point	Dilution	[Factor X] (µg/ml)
P1	1 part P1 (8 µg/ml) + 7 part Diluent	1.000
P2	1 part P1 + 3 part Diluent	0.250
P3	1 part P2 + 3 part Diluent	0.0625
P4	1 part P3 + 3 part Diluent	0.0156
P5	Diluent	0.000

6. Factor Xa Substrate: Add 1.1 ml of reagent grade water. Allow the substrate to sit for 10 minutes with gentle agitation prior to use, keep the vial on ice. Any remaining solution should be frozen at -20°C or below and used within 10 days. Avoid repeated freeze-thaw cycles.

9. Assay Method

1. Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at 37^0C in a humid incubator to avoid evaporation for chromogenic activity assay. Seal the plate with sealing tape at each step.

2. Remove excess microplate strips from the plate frame.

3. Add 20 µl of Factor X Standard or diluted sample to each well.

4. Assay Mix: Freshly prepare the desired volume of the Mix by combining the following reagents according to the assay numbers (n) plus one.

Assay Mix Reagent	n = 1 zell
Assay Diluent	10 µl
RVV	10 µl
Factor Xa Substrate	20 µl

5. Add 40 µl of above Assay Mix to each well. Read the absorbance at 405 nm at zero minutes for background O.D.

Seal the plate with sealing tape and incubate at 37^0C in a humid incubator.

- For high level of Factor X activity, read the absorbance at 405 nm every 5 minutes for 20 minutes.
- For low level of Factor X activity, read the absorbance at 405 nm every 5 minutes for 80 minutes.

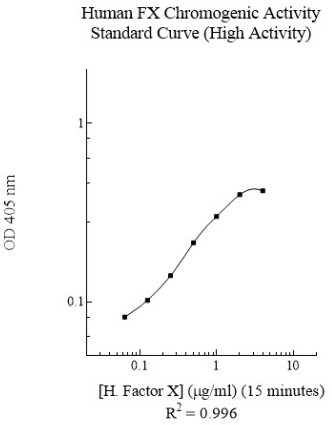
Incubate microplate at 37°C after each reading.

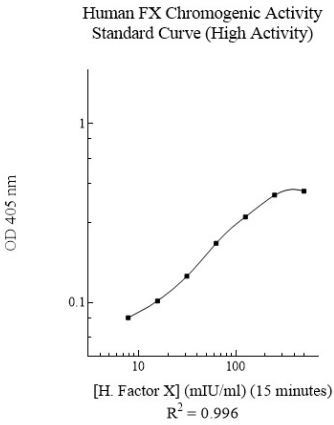
10. Data Analysis

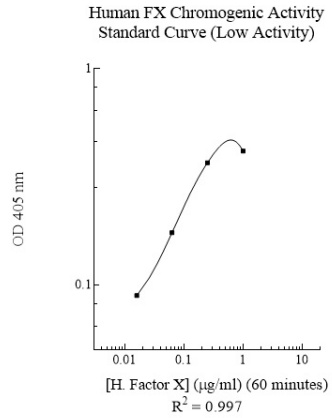
Calculate the mean value of the duplicate or triplicate for each standard and sample. To generate a Standard Curve from the optimalreaction time, plot the graph using the standard concentrations on the x-axis and the corresponding mean 405 nm absorbance or change in absorbance per minute (ΔA/min) on the y-axis. The best-fit line can be determined by regression analysis of the 4-parameter curve. Subtract the zero minutes background OD from corresponding unknown sample OD. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

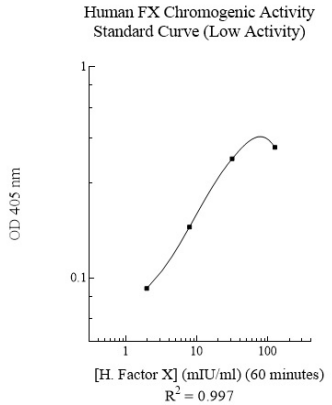
Typical Data

The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.









11. Performance Characteristics

- Kit standard has been calibrated against WHO International Standard.
- The minimum detectable dose of human FX at 60 minutes is approximately 9 ng/ml.
- No other enzyme that activates the substrate in plasma was observed.

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)