

ROX FACTOR XIa – 11 00 50

For In Vitro Research Use – Not For Diagnostic Use

Rossix

1 INTENDED USE

Quantitative activity determination of contaminating Factor XIa (FXIa) in enriched and highly purified protein preparations. Not intended for analysis of patient plasma.

2 BIOCHEMISTRY

Factor XI (FXI) is a dimeric protein of 160 kDa, which is activated to FXIa by FXIIa or by thrombin. Activation of FXI may occur inadvertently during protein purifications and FXIa can be a contaminant in intermediate or final products.

3 MEASUREMENT PRINCIPLE

FXIa functional activity is determined in a chromogenic method. FXIa in the sample activates human FIX to FIXa in the presence of calcium ions. Generated FIXa activates human FX in the presence of human FVIII, calcium ions and phospholipid. The amount of activated FX is determined from the hydrolysis of a chromogenic FXa substrate and is related to the FXIa activity of the sample.

The concentration of functionally active FXIa is assigned vs. a FXIa Calibrator and expressed in Units.

4 KIT COMPOSITION

Reagent 1 (2 vials) – REF 1110

Reagent 1 contains lyophilized human Factor IX, human Factor VIII and calcium chloride.

Each vial is sufficient for 50 tests.

Reagent 2 (2 vials) – REF 1120

Reagent 2 contains lyophilized human Factor X, bovine thrombin, calcium chloride and phospholipids.

Each vial is sufficient for 50 tests.

FXa Substrate, 6 mL (1 vial) – REF 9080

Liquid solution of chromogenic FXa substrate (Z-D-Arg-Gly-Arg-pNA), 2.5 mmol/L, containing a thrombin inhibitor.

Ready to use.

Diluent Buffer, Stock Solution, 20 mL (1 vial) – REF 1150

Liquid stock solution of diluent buffer.

5 PRECAUTIONS AND WARNINGS

The reagents are matched – only use reagents and diluent buffer from the same kit lot.

CAUTION: Each donor unit used in the preparation of Reagent 1 and Reagent 2 has been tested by FDA approved methods for the presence of Hepatitis B surface antigen and antibodies to HIV 1 and 2 and Hepatitis C and found to be negative. However, since no test can completely rule out the presence of these blood borne diseases, the handling and disposal of this human source reagent should be made with care.

- Avoid contact with skin and eye.
- Do not empty into drains.
- Wear suitable protective clothing.

6 PREPARATION

Reagent 1

Reconstitute with **3.0 mL** water. Allow to stand for 5 min at 20-25°C with intermittent gentle mixing for complete reconstitution.

Reagent 2

Reconstitute with **3.0 mL** water. Allow to stand for 5 min at 20-25°C with intermittent gentle mixing for complete reconstitution.

FXa Substrate, 6 mL

Ready for use.

Diluent Buffer, Stock Solution, 20 mL

Before use, dilute 1 + 9 with water to obtain a 0.05 mol/L Tris-HCl buffer working solution, pH 7.9 with 1% bovine serum albumin.

Note: All reconstitutions and dilutions of kit reagents and diluent shall be made with water of a quality of at least NCCLS Type II water or Ph. Eur. water for injection.

7 STORAGE AND STABILITY

The sealed reagents are stable at 2-8°C until the Expiry Date printed on the label. Be careful to avoid contamination of the reagents by microorganisms.

- **Reconstituted Reagent 1 and Reagent 2:**
Stability after reconstitution is 8 hours at 2-8°C, 2 hours at 20-25°C and 2 hours at 37°C.
- **Chromogenic FXa substrate:**
Opened vial is stable for 1 month at 2-8°C.
- **Diluent Buffer:**
Stock Solution: Opened vial is stable for 1 month at 2-8°C.
Buffer working solution should be used the same day as prepared.

8 MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized water, NCCLS Type II water or Ph Eur water for injection or higher quality.
- Citric acid, 2% (for end-point method)
- Calibrated pipettes
- Photometer, 405 nm (and 490 nm for end-point method)
- Heat incubator 37°C
- Plastic test tubes and Vortex mixer
- Stop-watch
- Low binding microplate (do not use high binding microplates intended for ELISA)

9 SYMBOLS USED



Catalog number



Batch code



Use by



Temperature limitation



Consult instruction for use



Biological risks



Manufacturer

10 METHOD

10.1 Standard dilutions

A calibration curve should be included in each run.

Prepare standard dilutions in Diluent Buffer working solution to obtain standards in the range 0.04–10 mIU/mL. Prepare at least five different standard dilutions. All dilutions should be prepared in plastic test tubes. Note that the unit "IU" for FXIa has no relation to the unit "IU" for FXI.

The calibration curve could be limited to the range 0.04 – 1.4 mIU/mL. When using this restricted range, a quadratic curve fit could be used.

Example:

Predilute the reconstituted FXIa Calibrator in Diluent Buffer working solution to a potency of 10 mIU/mL and prepare further dilutions according to the table below. It is recommended to prepare independent predilutions for each standard.

Preparation of FXIa Calibration curve, RANGE 0.04 – 10 mIU/mL		
FXIa mIU/mL	Volume of FXIa calibrator	Volume of Diluent Buffer working solution
10	1000 µL of 10 mIU/mL FXIa	-
4	200 µL of 10 mIU/mL FXIa	+ 300 µL
1,4	100 µL of 10 mIU/mL FXIa	+ 614 µL
0,4	50 µL of 10 mIU/mL FXIa	+ 1200 µL
0,1	20 µL of 10 mIU/mL FXIa	+1980 µL
0,04	200 µL of 0.1 mIU/mL FXIa	+ 300 µL
0	-	500 µL

NOTE: The above table is an example only and is based on a FXIa calibrator with an activity of 10 mIU/mL.



Rossix AB
Taljegårdsgatan 3B
SE-431 53 Mölndal, Sweden

info@rossix.com
www.rossix.com

ENGLISH – Insert Revision 04/2025

ROX FACTOR XIa – 11 00 50

For In Vitro Research Use – Not For Diagnostic Use

Rossix

10.2 Sample dilution

Dilute the sample e.g. 1:40 in Diluent Buffer working solution. Use the same lot of diluent buffer that was used for dilution of the calibrator. All dilutions should be prepared in plastic test tubes.

To obtain a higher sensitivity, a lower sample dilution (1:10 - 1:40) might be used. It is then necessary to check for any matrix interference by comparing assigned FXIa activities at several different dilutions.

In case of low or no measured FXIa activity, matrix interference may suitably be investigated by spiking the sample with e.g. 20 mIU/mL FXIa and determining the recovery in the sample when assayed at several different dilutions, starting at a 1:10 dilution. This will establish the minimal dilution required to eliminate matrix interference.

10.3 1188 Factor XIa Control – available as a separate product, not provided in the kit

Dilute the control 1:10 in Diluent buffer working solution. Use the same lot of diluent buffer that was used for dilution of the calibrator. All dilutions should be prepared in plastic test tubes.

10.4 Assay

Sample / Standard dilution (20-25°C) 50 µL

Heating 3-4 min, 37°C

Reagent 1 (37°C) 50 µL

FIX activation 4 min, 37°C

Reagent 2 (37°C) 50 µL

FIX and FX activation 2 min, 37°C

FXa Substrate (37°C) 50 µL

Kinetic method: Read $\Delta A_{405}/\text{min}$ at 37°C

End-point method: Incubate at 37°C for 2 min

Citric Acid, 2% (Endpoint method only) 50 µL

Kinetic method:

Read the absorbance change at 405 nm ($\Delta A_{405}/\text{min}$). A lag time may be introduced before starting the data acquisition.

End-point method:

Stop the reaction with 2% citric acid after 2 min hydrolysis at 37°C. Read the absorbance at 405 nm, using 490 nm as reference wavelength. Absorbance readings should be made within 2 hours after termination of the substrate hydrolysis.

11 CALCULATION

Plot the maximal absorbance change/minute ($\Delta A_{405\text{max}}/\text{min}$) or absorbance ($A_{405-490}$) vs. log FXIa activity.

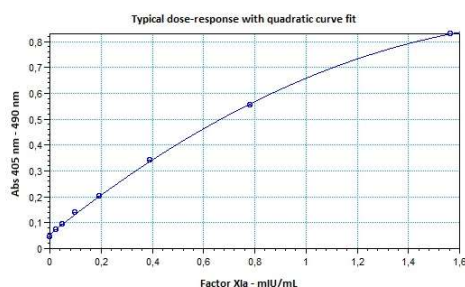
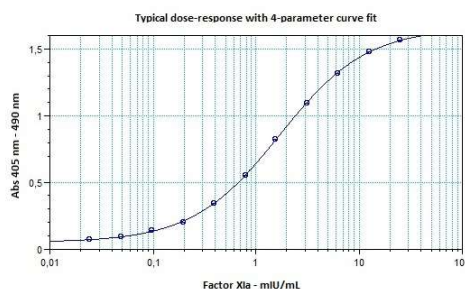
Use a 4-parameter curve fit, $y = ((A-D)/(1 + (x/C)^B))+D$.

Alternatively, when using a standard range up to 1.4 mIU/mL:

Plot absorbance change/minute ($\Delta A_{405\text{max}}/\text{min}$) or absorbance ($A_{405-490}$) vs. FXIa activity

Use a quadratic curve fit, $y = A + Bx + Cx^2$

- The FXIa activity of the tested sample is obtained directly from the calibration curve. Correct the obtained value with the dilution factor used.
- If several sample dilutions are used, adjust for each dilution with the appropriate dilution factor.
- Express the FXIa result as IU/mL.



12 PERFORMANCE CHARACTERISTICS

Sensitivity:	The assay allows detection of about 0.03 mIU/mL FXIa activity, which translates to detection of 1.2 mIU/mL in the neat sample when using a sample dilution of 1:40.
Imprecision:	Intra-assay CV < 4% at 1.6 mIU/mL FXIa < 4% at 0.08 mIU/mL FXIa Inter-assay CV < 3% at 1.6 mIU/mL FXIa < 5.5% at 0.08 mIU/mL FXIa
Imprecision data were obtained from 6 assay series using 6 replicates of each concentration.	

13 INTERFERENCE

Assuming a recommended sample dilution of 1:40, Factor XIa results are not affected by the following levels in the neat sample of the analytes stated below:

Ethanol	≤ 50%
NaCl	≤ 1 M
Factor XI zymogen:	No effect at 0.2 µM
Human kallikrein:	≤ 50 nM
Factor II	≤ 0.2 µM
Factor X:	No effect at 0.5 µM
Factor Xa:	< 1.2 nM
Factor IXa:	≤ 1 mIU/mL

No synergistic effects were observed in mixtures of human kallikrein in the absence or presence of human FXI and/or human FXIa.

14 REFERENCES

1. M-E. Behr-Gross, D. Le Tallec, N. Sinitskaya, C. Milne, M. Etscheid. Determination of procoagulant activity in human normal immunoglobulin preparations for therapeutic use by FXIa chromogenic assay: Evaluation of test kit sensitivity, reference standard performance and product formulation effects on the FXIa assay. *Pharmeuropa Bio & Scientific Notes*, March (2024)
2. Wilmot HV, Gray E. Enabling accurate measurement of activated factor XI (FXIa) in therapeutic immunoglobulin products. *Int. J. Clin. Transfus. Med* 166(6), 656-664 (2021).
3. Rosén S. New Tools to Study Contact Activation. *Front. Med.* 3, 58 (2016).
4. Roemisch J, Zapfl C, Zochling A, Pock K. Comparison of Thrombin Generation Assay (TGA) and Non-Activated Partial Thromboplastin Time (NAPTT) for the assessment of enhanced procoagulant activity in immunoglobulin solutions. *WebmedCentral IMMUNOTHERAPY* 3(4):WMC003247 (2012).
5. Etscheid M, Breitner-Ruddock S, Gross S, Hunfeld A, Seitz R, Dodt J. Identification of kallikrein and FXIa as impurities in therapeutic immunoglobulins: implications for the safety and control of intravenous blood products. *Vox Sanguinis* 102, 40-46 (2012).



Rossix AB
Taljegårdsgatan 3B
SE-431 53 Mölndal, Sweden

info@rossix.com
www.rossix.com

ENGLISH – Insert Revision 04/2025