

Rox Factor Xla

Art No. 110050

For In Vitro Research Use - Not for Diagnostic Use

2 x 50 tests / kit

Rox Factor XIa

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1 INTENDED USE

Quantitative activity determination of contaminating Factor XIa (FXIa) in enriched and highly purified protein preparations. Not intended for analysis of 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, in which human FIX is activated to FIXa in the presence of calcium ions. FIXa then activates human FX in the presence of human FVIII, calcium ions and phospholipid. The amount of FXa 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) - 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) - 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) - 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) - 1150

Liquid stock solution of diluent buffer.

5 PRECAUTIONS AND WARNINGS

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 (1110)

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 (1120)

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 (9080)

Ready to use.

Diluent Buffer, Stock Solution, 20 mL (1150)

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 should be made with water of a quality of at least NCCLS Type II water.

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 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 CALIBRATOR AND CONTROL

FXIa Calibrator (Art No. **1199**) and FXIa Control (Art No. **1188**) are available from Rossix in packages of 10 vials each. Both products are assigned in milliUnits and calibrated against NIBSC reference reagent for Activated Blood Coagulation Factor XI (FXIa), Human, 11/236.

9 MATERIALS REQUIRED BUT NOT PROVIDED

- Deionized water, filtered through 0.22 µm or NCCLS Type II water 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
- Stop-watch
- Vortex mixer

10 SYMBOLS USED



Catalog number



Batch code



Use by

Temperature
limitationConsult
instruction for
use

Biological risks



Manufacturer

11 METHOD

11.1 Standard dilutions

A standard curve should be included in each run.

Prepare standard dilutions in Diluent Buffer working solution to obtain standards in the range 0.04–10 mU/mL. Prepare at least five different standard dilutions. All dilutions should be prepared in plastic test tubes.

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

Example:

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

| Preparation of FXIa Standard dilutions, RANGE 0.04 – 10 mU/mL | | |
|---|--------------------------|--|
| FXIa Standard, mU/mL | Volume of FXIa Standard | Volume of FXIa Diluent Buffer working solution |
| 10 | 1000 µL of 10 mU/mL FXIa | - |
| 4 | 200 µL of 10 mU/mL FXIa | + 300 µL |
| 1.4 | 100 µL of 10 mU/mL FXIa | + 614 µL |
| 0,4 | 50 µL of 10 mU/mL FXIa | + 1200 µL |
| 0,1 | 20 µL of 10 mU/mL FXIa | +1980 µL |
| 0,04 | 200 µL of 0.1 mU/mL FXIa | + 300 µL |
| 0 | | 500 µL |

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

11.2 Sample dilution

Dilute the sample 1:40 in Diluent Buffer working solution. All dilutions should be prepared in plastic test tubes.

To obtain an even 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 mU/mL FXIa and determine 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.

11.3 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.

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.

12 CALCULATION

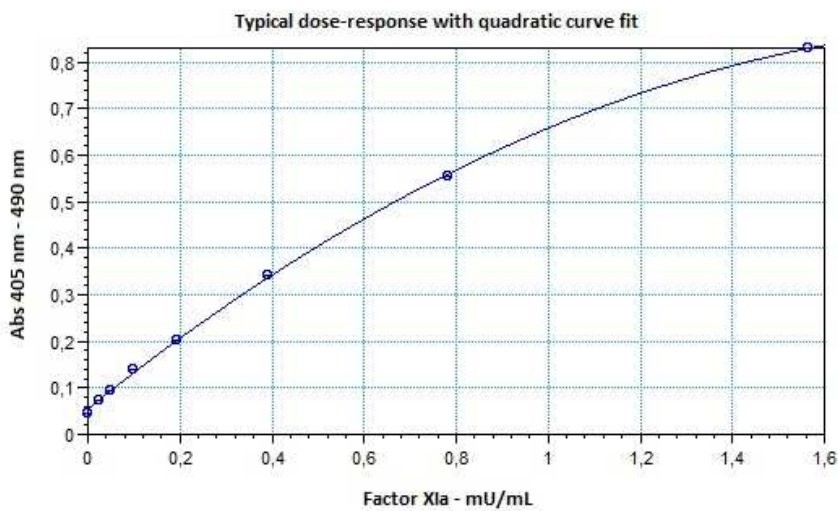
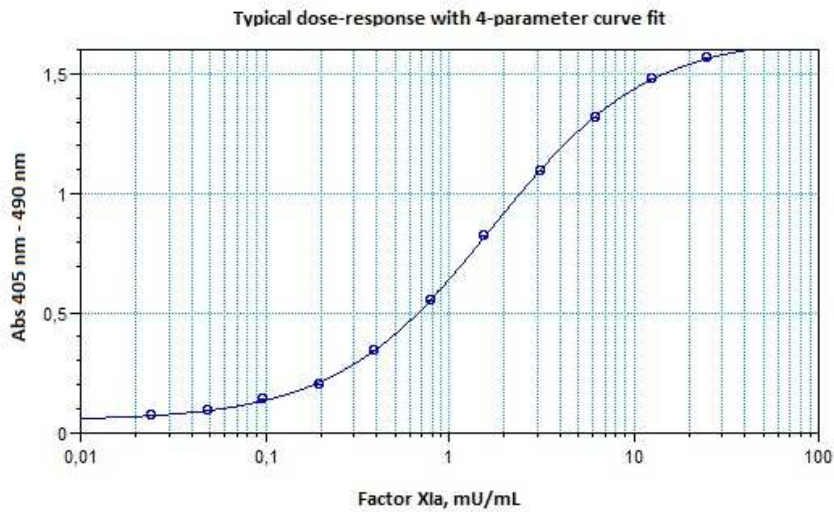
- Plot absorbance change/minute ($\Delta A_{405}/\text{min}$) or absorbance ($A_{405-490}$) vs. log FXIa activity.
Use a 4-parameter curve fit, $y = ((A-D)/(1 + (x/C)^B))+D$.

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

Plot absorbance change/minute ($\Delta A_{405}/\text{min}$) 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 U/mL.



13 PERFORMANCE CHARACTERISTICS

Sensitivity: The assay allows detection of about 0.03 mU/mL FXIa activity, which translates to detection of 1.2 mU/mL in the neat sample when using a sample dilution of 1:40.

Imprecision:

Intra-assay CV
< 4% at 1.6 mU/mL FXIa
< 4% at 0.08 mU/mL FXIa

Inter-assay CV
< 3% at 1.6 mU/mL FXIa
< 5.5% at 0.08 mU/mL FXIa

Imprecision data were obtained from 6 assay series using 6 replicates of each concentration.


14 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.

15 CONTACT INFORMATION

| | | |
|---|--------------------|--|
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16 REVISION DATE

Last revision date: September 17, 2012

17 REFERENCES

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