Rox Factor XIa

Art. No. 110050
2 x 50 test

A chromogenic kit for quantitative activity determination of contaminating Factor XIa in enriched and highly purified protein preparations.

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Background

Factor XI (FXI) has been identified as a risk factor for both arterial and venous thromboembolism (Ref 1-3). Plasma fractionation procedures may sometimes be associated with inadvertent activation of hemostasis zymogen proteins such as FXI and Factor XIa (FXIa) can be a contaminant in intermediate or final products such as immunoglobulins (IgG). Thromboembolic events have been reported to occur after intravenous administration of IgG and ensuing detailed analyses appeared to link these events to the presence of FXIa in the administered IgG batches (4-7). Routine tests such as the NAPTT may not be sufficiently sensitive to detect relevant low levels of FXIa, possibly partly explained by matrix interference. Rox Factor XIa offers a highly sensitive chromogenic method for determination of sub-picomolar levels of FXIa, therewith allowing high sample dilutions and minimizing interference from the sample matrix and from contaminating proteins such as kallikrein, zymogen FXI and FIXa.

Intended Use

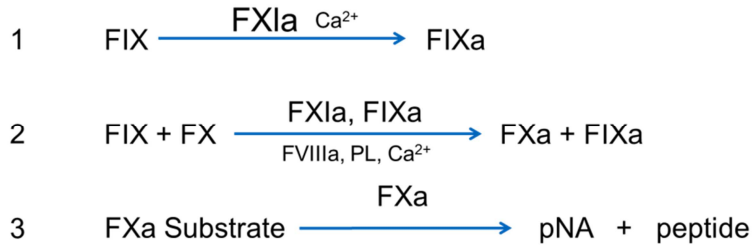
Quantitative activity determination of contaminating Factor XIa (FXIa) in enriched and highly purified protein preparations. Not intended for analysis of plasma.

Measurement Principle

Human FXIa is determined from its activation of human FIX (8,9) and ensuing activation of human FX.
Generated FXa is then measured with a chromogenic FXa substrate (10).
The activation of FIX is performed in two steps:

1. Initial activation of FIX in the absence of phospholipids.
2. Continued activation of FIX in the presence of FX and phospholipids, therewith allowing concomitant activation of FX.

By using an initial activation step of FIX in the absence of phospholipids and hence with no FX activation, the sensitivity towards FXIa is considerably increased and the interference by contaminating FIXa in the sample is several-fold reduced.



Kit Components

Reagent 1 (2 vials) - Contains lyophilized human Factor IX, human Factor VIII and calcium chloride
Stability after reconstitution is 8 hours at 2-8°C and 2 hours at 37°C.

Reagent 2 (2 vials) - Contains lyophilized human Factor X, bovine thrombin, calcium chloride and phospholipids.
Stability after reconstitution is 8 hours at 2-8°C and 2 hours at 37°C

FXa Substrate, **6 mL** (1 vial) - Liquid solution of chromogenic FXa substrate (Z-D-Arg-Gly-Arg-pNA), 2.5 mmol/L, containing a thrombin inhibitor. Ready to use. Opened vial is stable for 1 month at 2-8°C.

Diluent Buffer, Stock Solution, 20 mL (1 vial) - Liquid stock solution of Tris-HCl with BSA.
Before use, dilute 1 + 9 with water to obtain a buffer working solution. To be used the same day as prepared.

Sample and Standard Dilutions

Standard range:	0.02 - 2 pM
Recommended sample dilution:	1:40 Lower sample dilution can be used, dependent on the sample composition.

Standard and sample dilutions should be prepared in Diluent Buffer working solution.

Calibrator and Control

A FXIa Calibrator (Art No. 1199) and a FXIa Control (Art No. 1188) assigned in U vs. the interim 1st WHO Reference Reagent for Factor Xia (11/236) will be available within short. Both products are provided in packages of 10 vials each.

Microplate Assay Procedure

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**
Read kinetically or incubate 2 min at 37°C	
Citric Acid, 2% (Endpoint method only)	**50 µL**

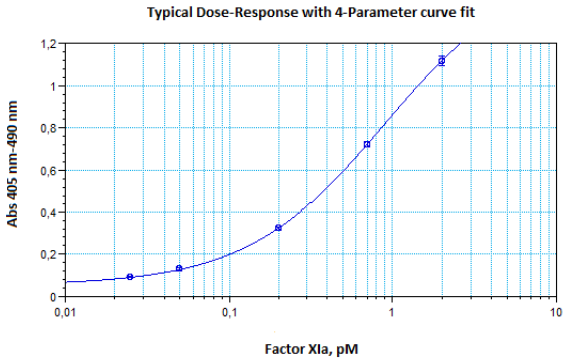
Kinetic method: Read the absorbance change at 405 nm.

End-point method: Read the absorbance at 405 nm, using 490 nm as reference wavelength.

Calculation

Plot absorbance change/minute (ΔA405/min) or absorbance (A405-490) for the standard dilutions vs. log FXIa activity. The FXIa activity of the tested sample is obtained directly from the calibration curve. Correct the obtained value with the dilution factor used.

A 4-parameter curve fit is recommended.



Performance Characteristics

Sensitivity: The assay allows detection of about 0.015 pM FXIa activity.

Imprecision: Intra-assay CV < 4% at 0.8 pM and < 4% at 0.04 pM FXIa (n = 24).

Inter-assay CV < 4% at 0.8 pM and < 6% at 0.04 pM FXIa.
FXIa assayed as six replicates in 5 series)

Interferences

Assuming a 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.

*Highest tested concentration.

References

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