

Rox Factor IX

Steffen Rosén, Rossix AB

FOR RESEARCH USE ONLY

Reagents

Reagent A

Lyophilized human FVIII, human FX, bovine FV and a fibrin polymerization inhibitor.

Reagent B

Lyophilized human FXIa, human FII, calcium chloride and phospholipids.

FXa Substrate

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

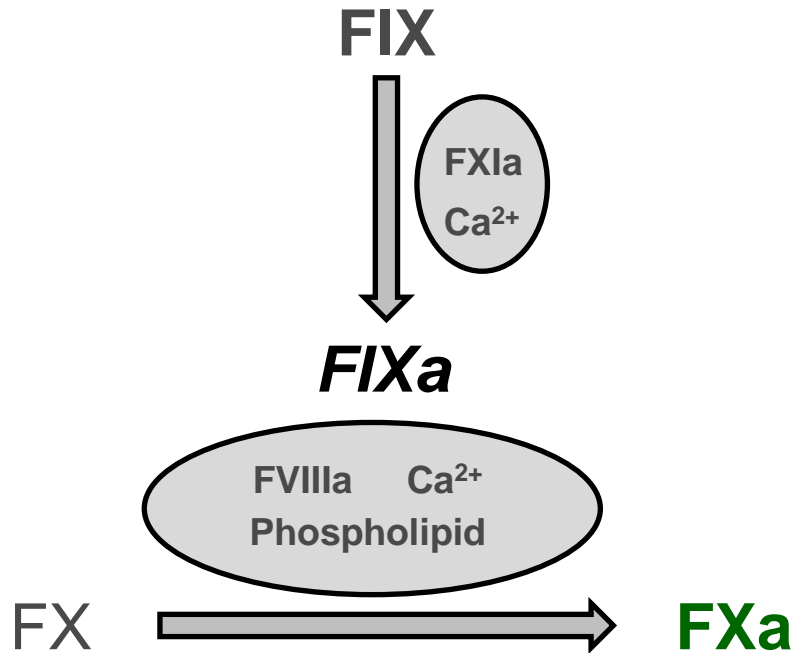
FIX Diluent Buffer, Stock Solution

Liquid stock solution of diluent buffer, containing a heparin antagonist.

No use of Factor IX deficient plasma

Method Principle

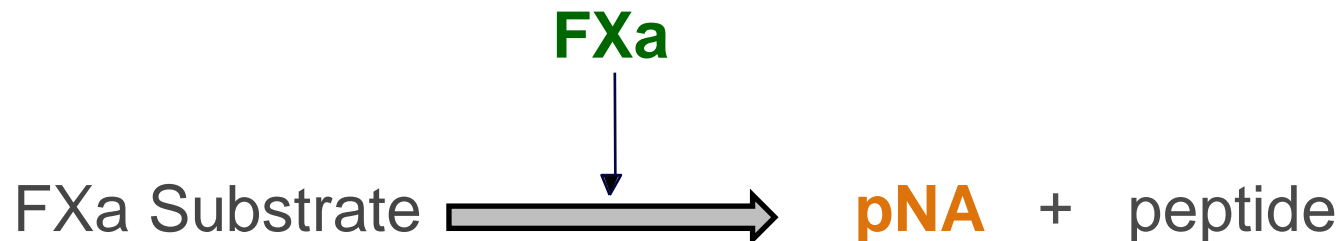
1



Activation of FIX and FX proceeds in parallel

Thrombin is rapidly generated in the assay through early formation of FXa and activates FV and FVIII

2



Manual Microplate End-Point Method

Sample Dilution (18-25°C)	25 µL
---------------------------	-------

Reagent A (18-25°C)	25 µL
---------------------	-------

Preheating, 3-5 min at 37°C

Reagent B (37°C)	150 µL
------------------	--------

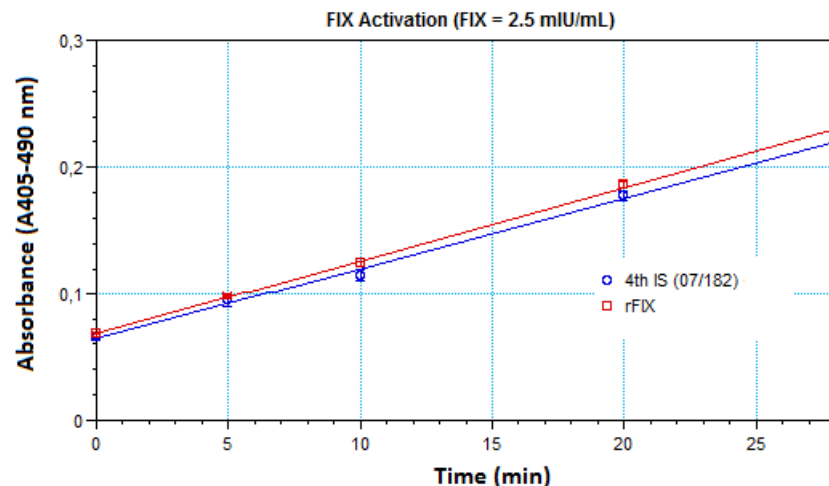
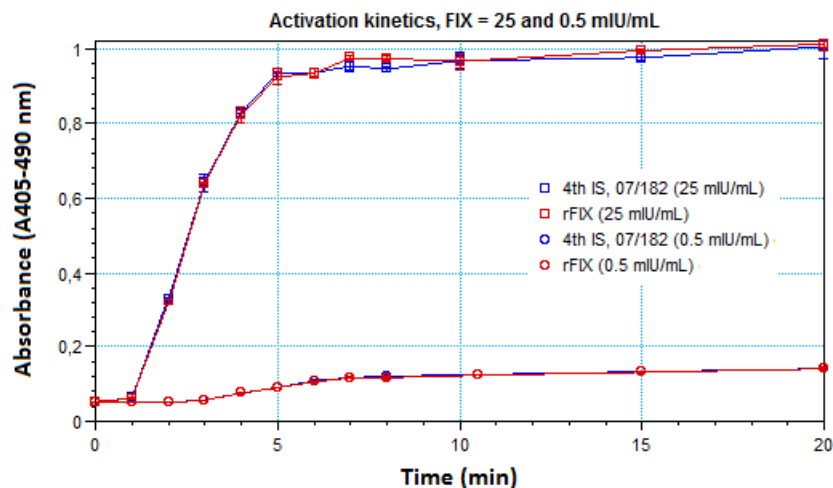
Activation, 8 min at 37°C

FXa Substrate (37°C)	50 µL
----------------------	-------

Hydrolysis, 2 min at 37°C

Citric Acid, 2% (18-25°C)	50 µL
---------------------------	-------

Method Principle – cont.

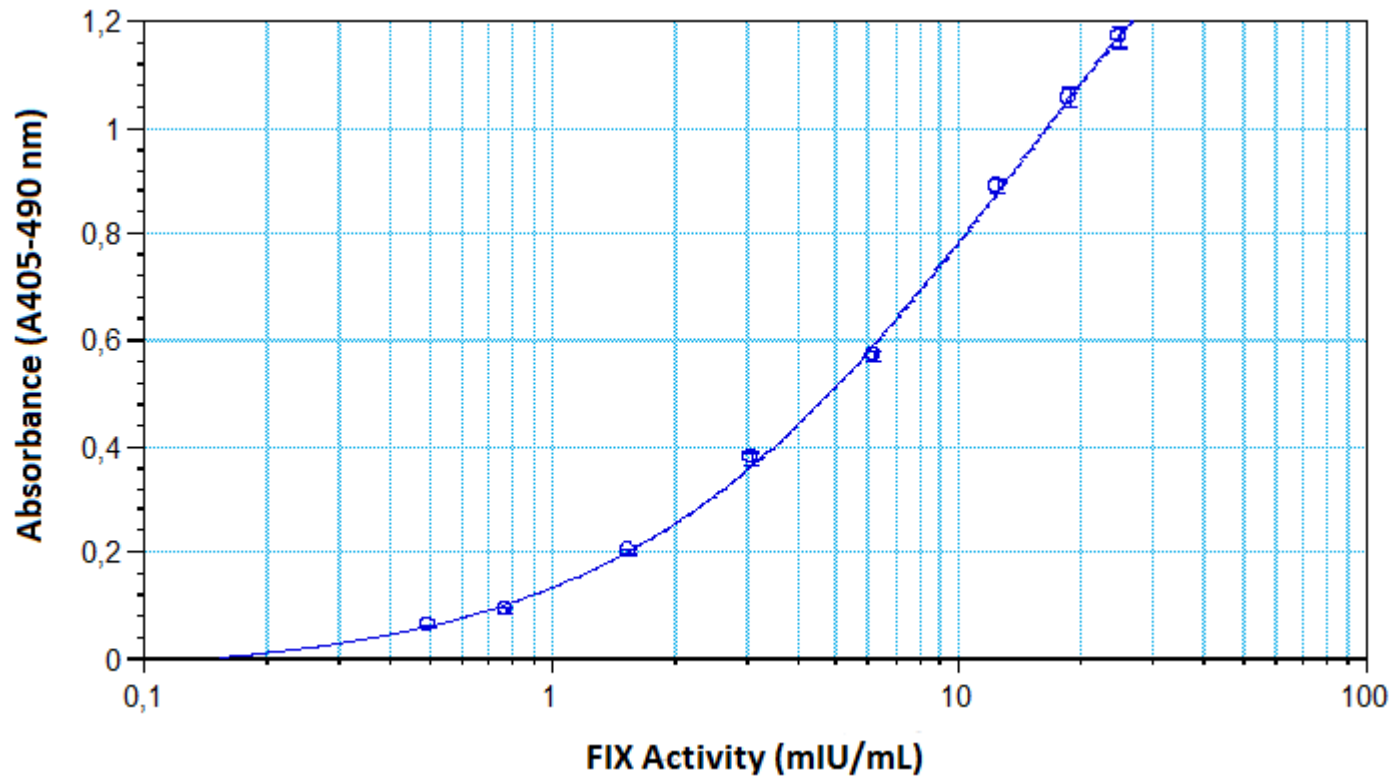


Mean assigned FIX potencies for rFIX and pdFIX vs 4th IS (07/182) at different activation times, using four dilutions for each sample.

Activation Time min	rFIX IU/mL (CV%)	pdFIX IU/mL (CV%)
2.5	90 (2.2%)	85 (4.1%)
4	90 (3.6%)	88 (3.6%)
8	88 (2.0%)	87 (4.1%)

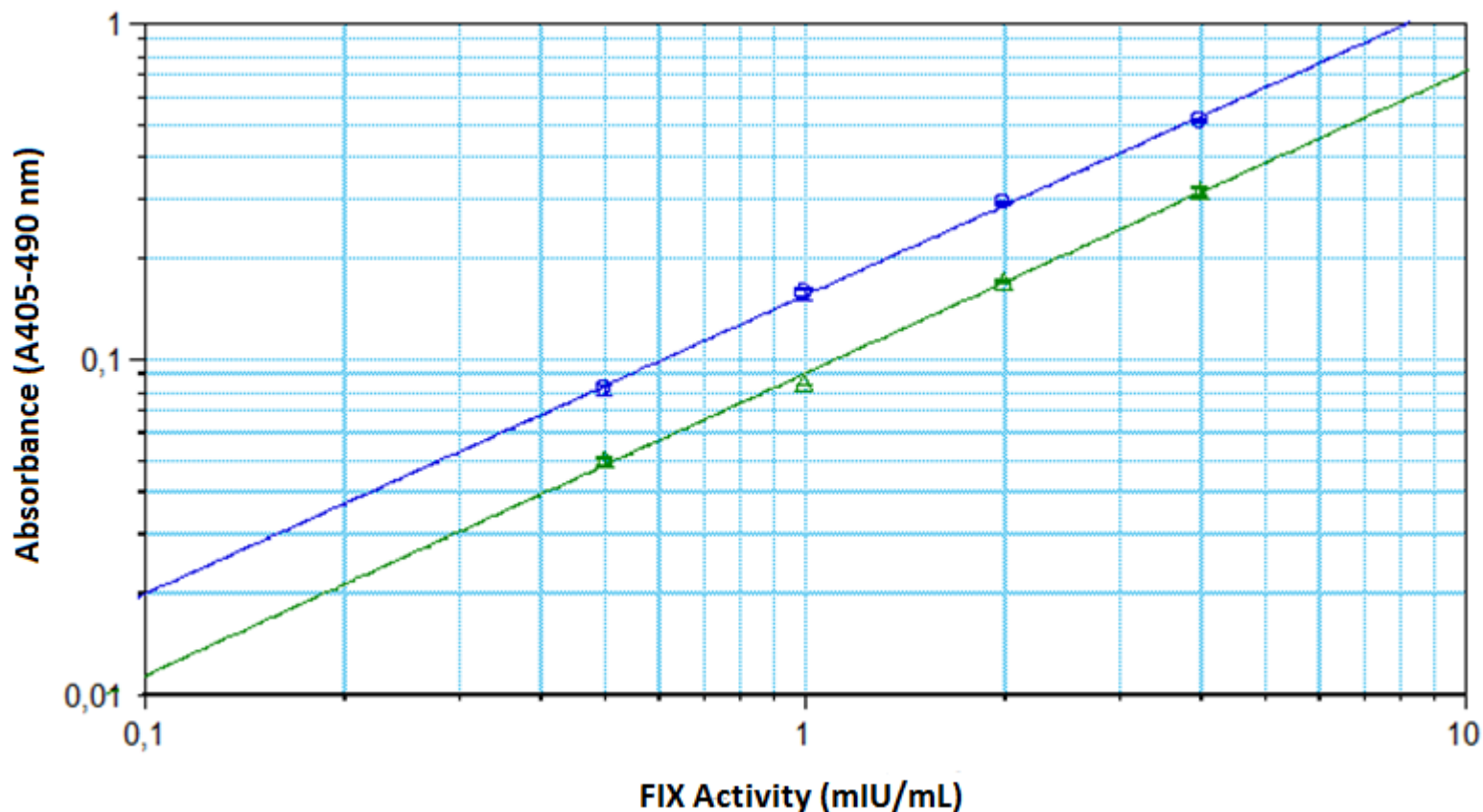
Dose Response

Typical dose response for a plasma standard in the range 0.5 - 25 mIU/mL (plasma diluted 1:2000 – 1:40) presented in a Semi-Log graph

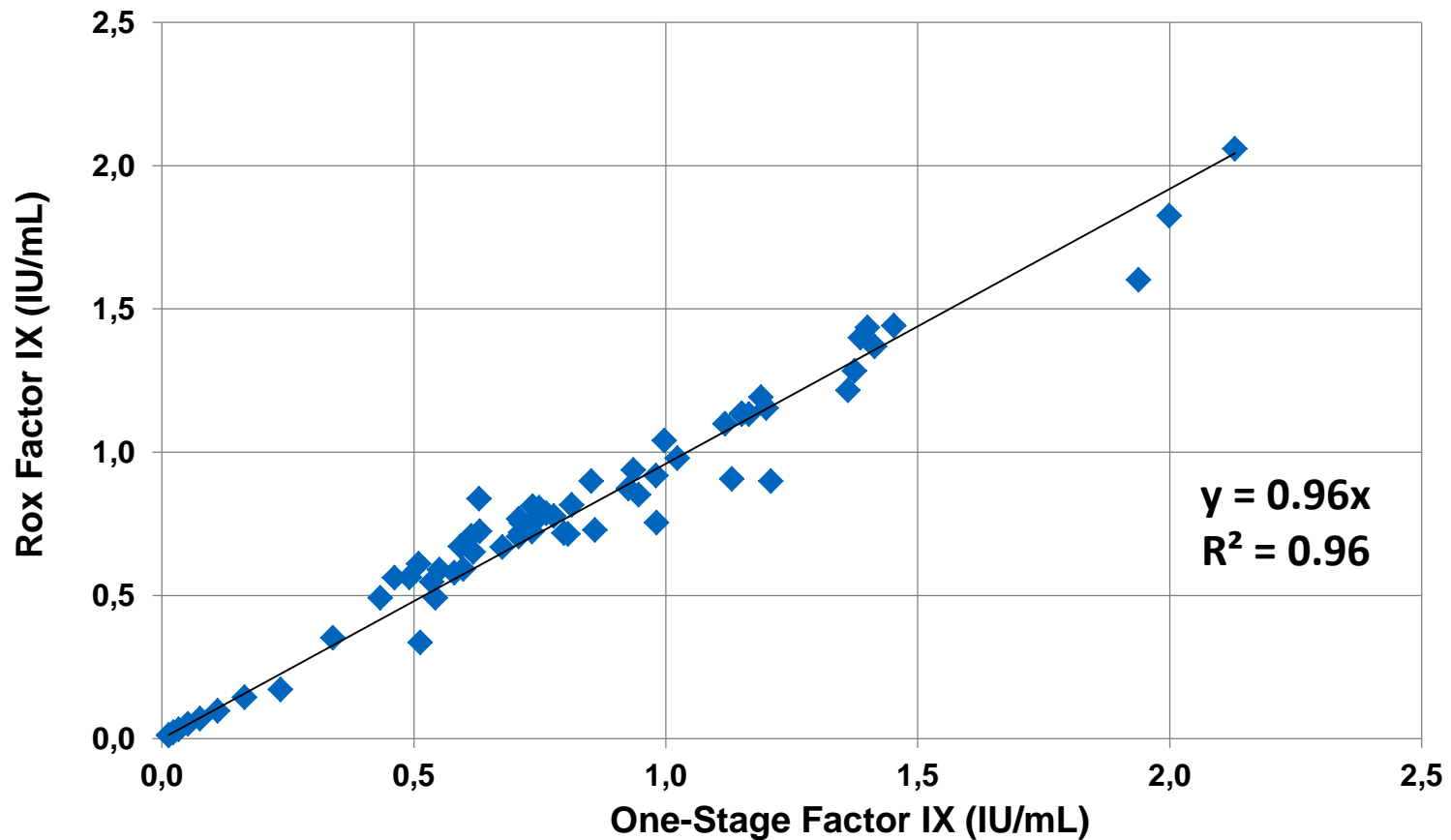


Parallel Line

Parallel line representation of a pdFIX concentrate sample vs. the 4th IS FIX Concentrate in the range 0.5 - 4 mIU/mL, using 4 min hydrolysis at 37°C

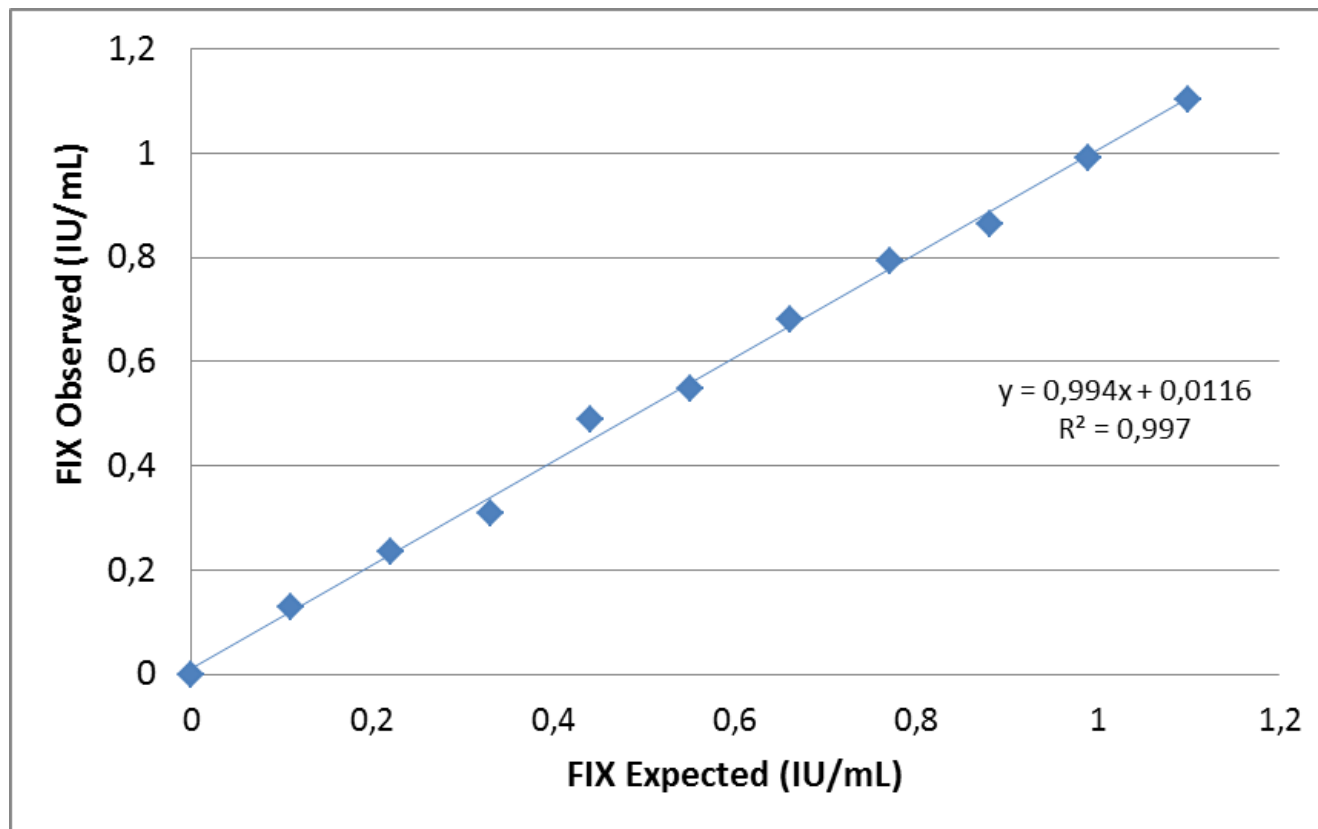


Correlation to One-Stage Clotting method



Dilutional Linearity

Dilutional linearity obtained on BCS XP



Discrimination at clinically relevant levels

Discrimination at relevant FIX deficiency classification levels using manual microplate method (N=5, n=20)

Sample	Mean \pm SD	\pm 2 SD
10 mIU/mL	10 \pm 1	8 - 12
15 mIU/mL	15 \pm 1	13 - 17
43 mIU/mL	43 \pm 2	39 - 47
53 mIU/mL	53 \pm 1	51 - 55
0.46 IU/mL	0.46 \pm 0.01	0.44 - 0.48
0.54 IU/mL	0.54 \pm 0.02	0.50 - 0.58

Limit of Detection about 0.25 mIU/mL

FIX determination in the absence and presence of FIX deficient plasma

Assigned activities of plasma samples calculated against a standard prepared in diluent \pm FIX deficient plasma (n=4).

Sample	Standard in diluent	Standard in diluent + 5% FIX def. plasma
10 mIU/mL	9.6 \pm 0.1	9.2 \pm 0.1
20 mIU/mL	20 \pm 0.4	20 \pm 0.4
53 mIU/mL	54 \pm 0.9	53 \pm 0.9
0.46 IU/mL	0.46 \pm 0.01	0.46 \pm 0.01

Interference

No interference by:

- Hemoglobin tested up to 5 mg/mL
- Bilirubin tested up to 0.4 mg/mL
- Triglycerides tested up to 5 mg/mL
- UF Heparin < 2 IU/mL

- Analyses in two laboratories have demonstrated no interference by Lupus Anticoagulant.

- No interference at 25 mIU FIXa/ 1 IU FIX

Thank you for your attention!