

Product information

Rox Factor VIII

REF 800070

2 x 100 tests

For In Vitro Research Use Only

Key characteristics:

- Excellent dilutional linearity over the whole measuring range, also at very low Factor VIII levels.
- Excellent discrimination at low Factor VIII activities.
- Conditions during activation are in agreement with the European Pharmacopoeia chapter 2.7.4 *Assay of human coagulation factor VIII*
- One range, only one calibration curve is used for the whole measuring range (0-200%). Normal sample dilution = 1:60, Samples with activity <5% are diluted 1:15.
- Stability of Reagents after reconstitution = 72 hours at 2-8°C.
- Reagents can be aliquoted and frozen at $\leq -70^{\circ}\text{C}$ in order to limit reagent waste.
- Not sensitive to Emicizumab (due to use of bovine Factor X instead of human Factor X in Reagent 1)
- Compared to FVIII kits with only bovine components, the Rox Factor VIII kit contains human FIXa which ensures proper interaction with human Factor VIII

Manufacturer

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Rossix AB operates an ISO 13485 Medical Devices Quality Management System certified by BSI under certificate number MD649316



1 INTENDED USE

Rox Factor VIII is a chromogenic kit for the determination of Factor VIII (FVIII) activity in human plasma and FVIII containing concentrates. This kit is for in vitro research use only and should not be used for patient diagnosis or treatment.

2 PERFORMANCE CHARACTERISTICS

Results obtained using the manual microplate method

Detection limit:

0.3% (0.003 IU/mL), calculated according to CLSI EP17-A using sample dilution of 1:15.

Quantification limit:

0.7% (0.007 IU/mL), calculated according to CLSI EP17-A using sample dilution of 1:15.

Precision:

Repeatability (Intra assay CV): **≤4%**

Within Laboratory (Inter assay CV): **≤4%**

The precision was determined at 5%, 90% and 130% Factor VIII activity.

Linearity:

0.5 - 200% (0.005 – 2 IU/mL), calculated according to CLSI EP06-A.

3 MEASUREMENT PRINCIPLE

In the presence of Ca^{2+} and phospholipids, Factor X is activated to Factor Xa by Factor IXa. This reaction is greatly stimulated by FVIII after activation to FVIIIa by thrombin. By using optimal concentrations of Ca^{2+} , phospholipids and an excess of Factor IXa, Factor X and thrombin, the rate of activation of Factor X is directly related to the amount of FVIII in the sample. Factor Xa hydrolyses the chromogenic Factor Xa substrate, Z-D-Arg-Gly-Arg-pNA, thus liberating the chromophoric group pNA. The colour is read photometrically at 405 nm and the generated FXa and thus the intensity of colour is proportional to the FVIII activity in the sample.

4 KIT COMPOSITION

Reagent 1 (2 vials)

Reagent 1 contains lyophilized bovine FX and a fibrin polymerization inhibitor.

Reagent 2 (2 vials)

Reagent 2 contains lyophilized human FIIa, human FIXa, calcium chloride and phospholipids.

FXa Substrate, 6 mL (2 vial)

Liquid solution of chromogenic FXa substrate (Z-D-Arg-Gly-Arg-pNA), 2.5 mmol/L, containing a thrombin inhibitor. Contains sodium azide $\leq 0.01\%$ ($\leq 0.1\text{g/L}$)

Tris BSA Buffer, Stock Solution, 20 mL (1 vial)

Liquid stock solution of diluent buffer, containing 10% Bovine Serum Albumin (BSA) and a heparin antagonist. Stock solution contains sodium azide $\leq 0.01\%$ ($\leq 0.1\text{g/L}$)

5 STORAGE AND STABILITY

The sealed reagents are stable at 2-8°C until the Expiry Date printed on the label. Opened vials must be handled with care to avoid contamination during use.

Homogenize the content gently before each use.

- **Reagent 1:** Stability after reconstitution is 72 hours (h) at 2-8°C, 24h at 15-25°C, 2h at 37°C and 12 months at ≤ -70°C.

- **Reagent 2:** Stability after reconstitution is 72 hours (h) at 2-8°C, 24h at 15-25°C, 2h at 37°C and 12 months at ≤ -70°C.

- **Chromogenic FXa substrate:**

Opened vial is stable for 12 months at 2-8°C, 12 months at <-20°C or

7 days at 18-25°C. If the substrate becomes yellow, it indicates the presence of a contaminant and the vial must be rejected.

- **Tris BSA Diluent Buffer**

Stock Solution: Opened vial is stable for 12 months at 2-8°C provided microbial contamination is avoided.

Buffer working solution should be used the same day as prepared.

6 MANUAL MICROPLATE METHOD

Sample / Standard dilution	50 µL
Reagent 1 (Preincubated at 37°C)	50 µL
Reagent 2 (Preincubated at 37°C)	50 µL
<i>Activation - Mix and incubate for 3 min at 37°C</i>	
FXa Substrate (Preincubated at 37°C)	50 µL
<i>Kinetic method: Read ΔA405/min at 37°C</i>	
<i>End-point method: Hydrolysis at 37°C for 5 min</i>	
Citric acid, 2% (End-point method only)	50 µL

Kinetic reading:

Read the absorbance at 405 nm and record the change in absorbance.

End-point method:

Stop the reaction with 2% citric acid. 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.

7 CALIBRATION CURVE ANDS SAMPLE DILUTION

Use a human plasma or FVIII concentrate, traceable to a WHO International Standard for FVIII activity.

The calibration curve may be prepared as below. If only samples with low FVIII activities are analyzed, 200% and 150% can be replaced with 10% and 5% standards (total dilution 1:600 and 1:1200 respectively). 10% and 5% corresponds to 2.5% and 1.25% when using sample dilution 1:15:

Preparation of FVIII Calibration curve, RANGE 0 - 200 %			
FVIII Standard %	Total Dilution	Volume	Tris BSA Buffer, working solution
Predilution	1:10	100 µL of plasma	900 µL
200%	1:30	100 µL of predilution	200 µL
150%	1:40	100 µL of predilution	300 µL
100%	1:60	100 µL of predilution	500 µL
50%	1:120	100 µL of predilution	1100 µL
20%	1:300	50 µL of predilution	1450 µL
0%		0	500 µL

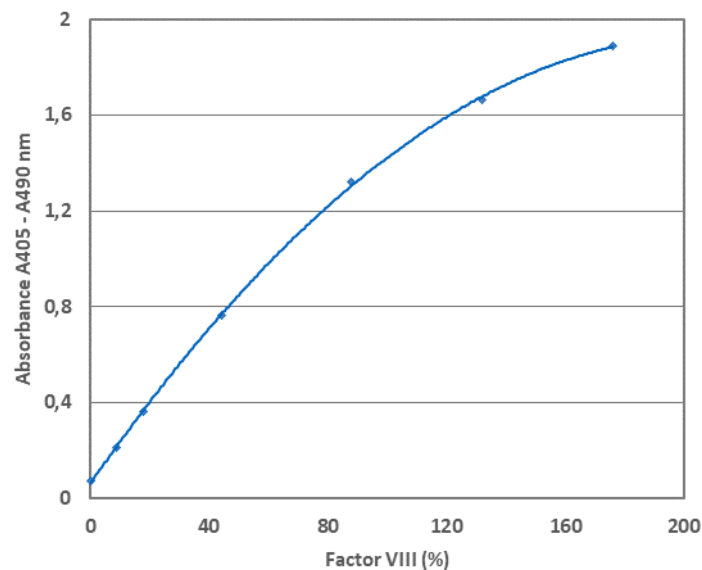
NOTE: 100% activity is defined as a FVIII activity of 1 IU/mL in plasma. In case the FVIII activity of the plasma standard differs from this value, the appropriate correction factor should be used when calculating the sample result. It is recommended to express all sample results as IU/mL.

Sample dilution - Samples with expected activity 5 - 200%

Plasma samples with an estimated potency of **5 - 200 %** (0.05 - 2 IU/mL) should be analysed using sample dilution **1:60**. The FVIII activity of the tested sample is obtained directly from the calibration curve.

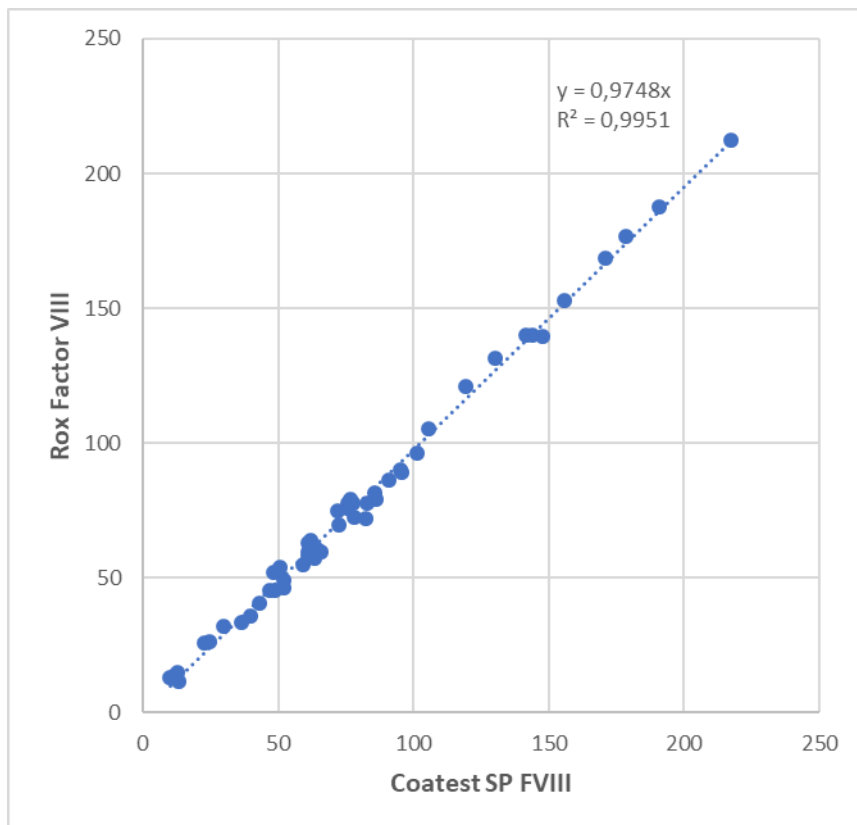
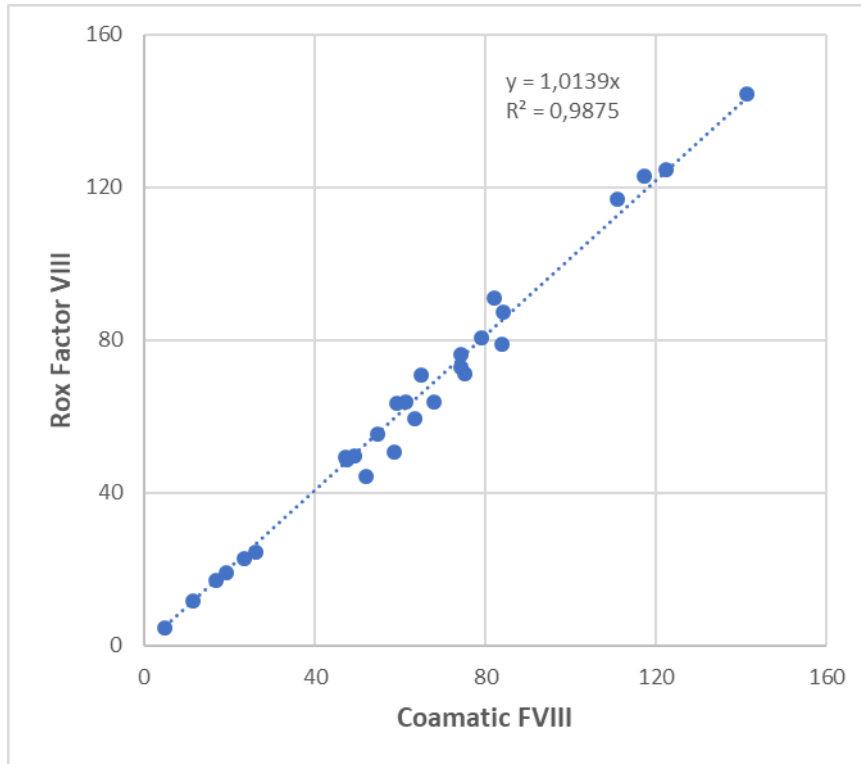
Sample dilution - Samples with expected activity 0 - 5%

Plasma samples with an estimated potency of **0 - 5%** (0 – 0.05 IU/mL) should be analysed using sample dilution **1:15**. **The FVIII activity of the tested sample is obtained by multiplying the results directly obtained from the calibration curve with the factor x0.25.**

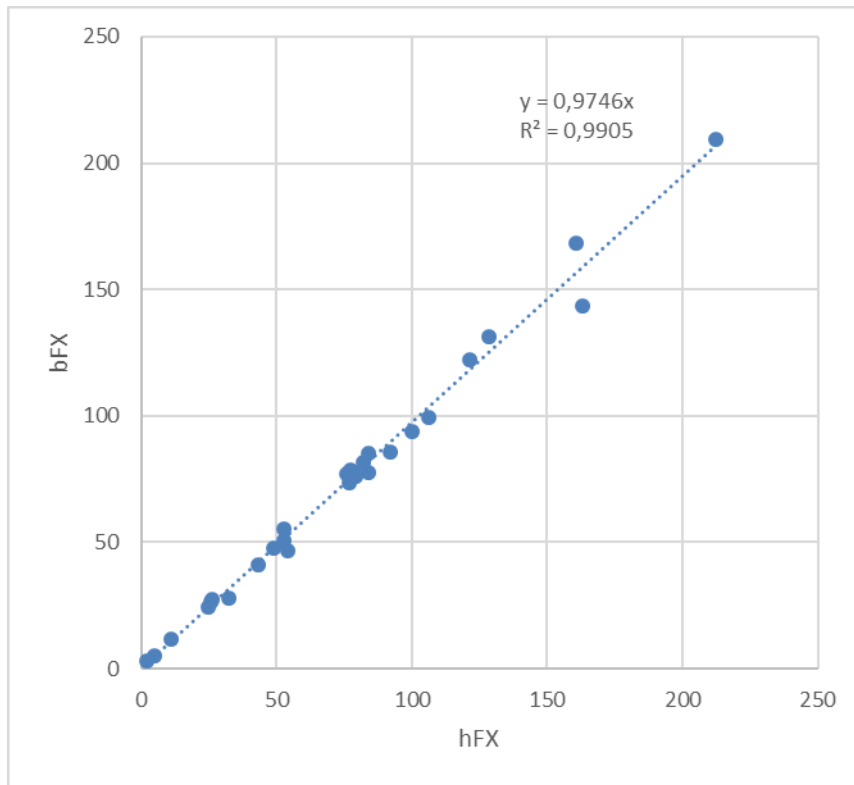


8 CORRELATIONS

Correlation, plasma samples, Rox Factor VIII vs. Coamatic FVIII and Coatest SP FVIII (Chromogenix)



Correlation, plasma samples, between Rox Factor VIII with bovine Factor X in Reagent 1 compared to using human Factor X in Reagent 1.

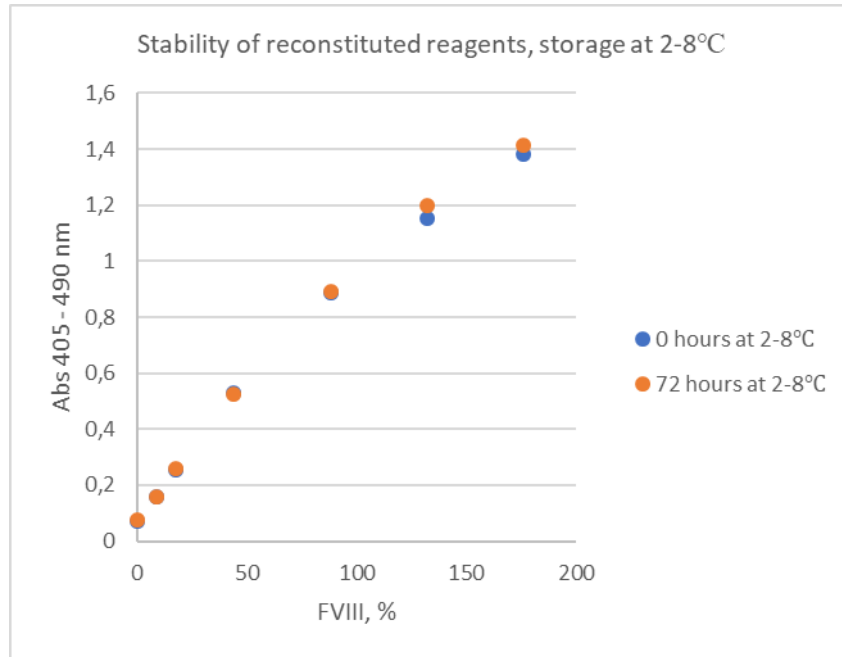


9 STABILITY

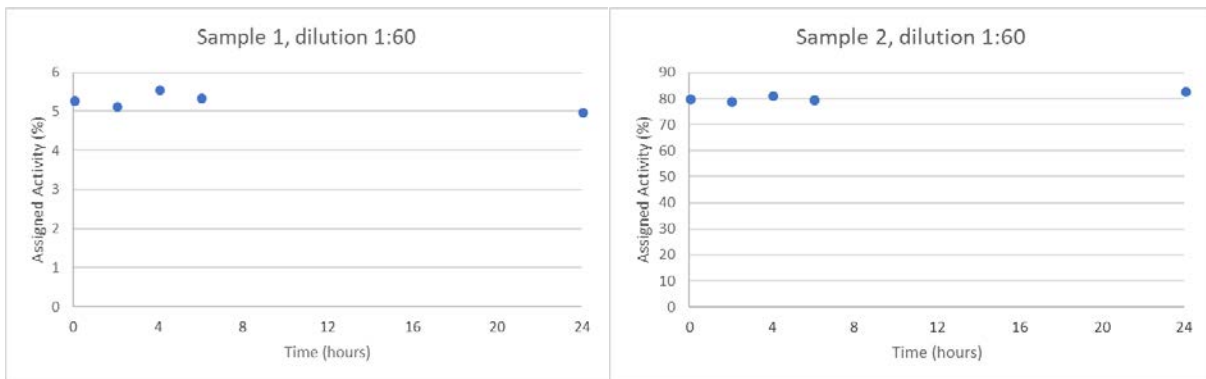
Special note:

In order to obtain the claimed stability it is important to homogenize the reagents prior to use if stored unstirred for > 8 hours.

In order to obtain a proper on board stability when using the reagents on an automated instrument it is important to use extensive washing/cleaning between pipetting of different reagents. A faster decay of activity over time than expected when stored on board indicates cross contamination between reagents due to insufficient cleaning.

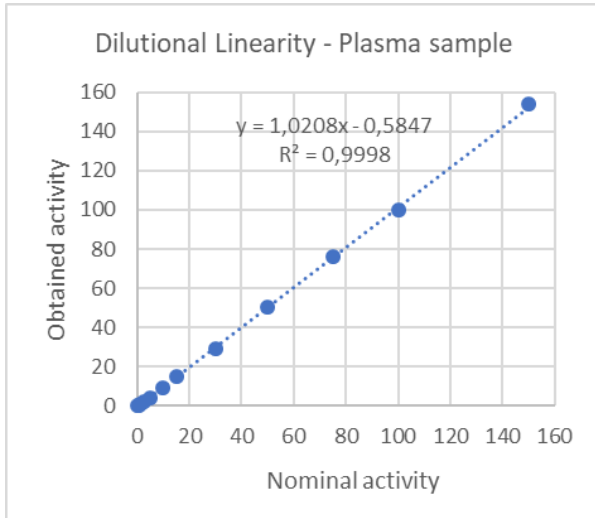


On board stability ACL TOP



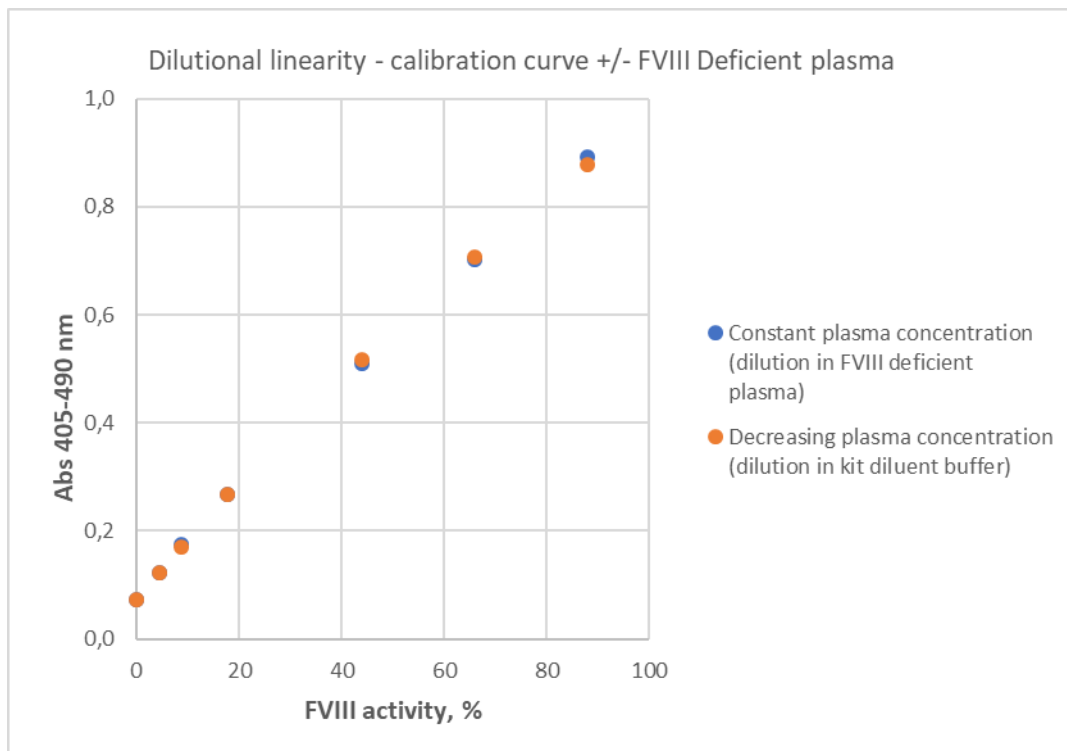
10 DILUTIONAL LINEARITY

A sample with nominal activity 150% was diluted in FVIII deficient plasma to obtain plasma samples with activities in the range 0.5 – 150%. Sample dilution 1:60 was used for samples 5-150% and sample dilution 1:15 was used for samples < 5%.



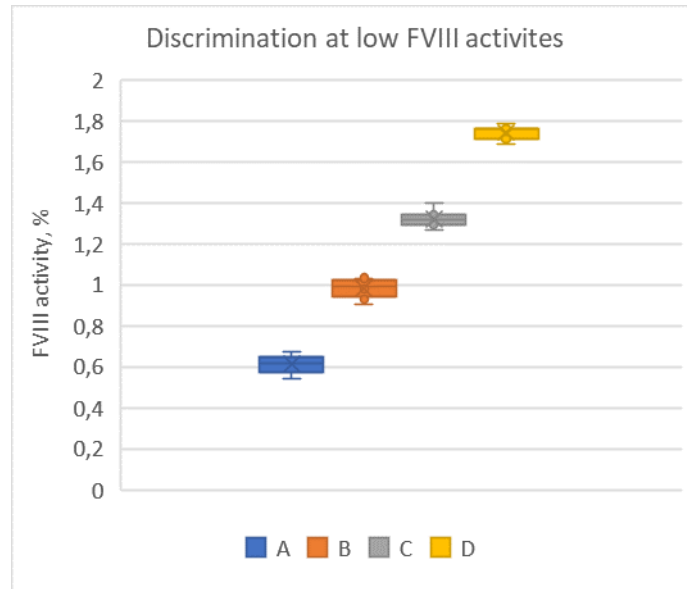
Nominal activity	Obtained Activity	Recovery
0,00	0,0	+∞
0,50	0,4	82,0%
1,00	1,0	96,0%
2,50	2,4	96,4%
5,00	4,3	85,2%
10,00	9,2	91,8%
15,00	14,8	98,5%
30,00	29,1	97,1%
50,00	50,2	100,4%
75,00	75,9	101,3%
100,00	100,0	100,0%
150,00	153,9	102,6%

A calibration plasma (SSC#4) was diluted in FVIII deficient plasma or the Rox Factor VIII kit diluent to obtain samples with activities 4.4 – 88% containing either a constant concentration of plasma or a gradual decrease in plasma concentration. All samples were then diluted 1:60 in kit diluent buffer, The results demonstrates that the results are not affected by the plasma matrix.



11 RESOLUTION AT LOW FVIII ACTIVITIES

Four plasma samples, A-D with FVIII activities < 2% were analysed in 8 replicates using sample dilution 1:15. The results demonstrates a good discrimination at the low end of the calibration curve.



12 ACTIVATION KINETICS

The Rox Factor VIII activation time of 3 min results in a FXa generation of about 50% of max and is in agreement with the European Pharmacopoeia chapter 2.7.4 that states: *“It is important to demonstrate by validation the suitability of the kit used, notably by checking the time course of factor Xa generation in order to determine the **time taken to reach 50 per cent** of the maximal factor Xa generation.”*

