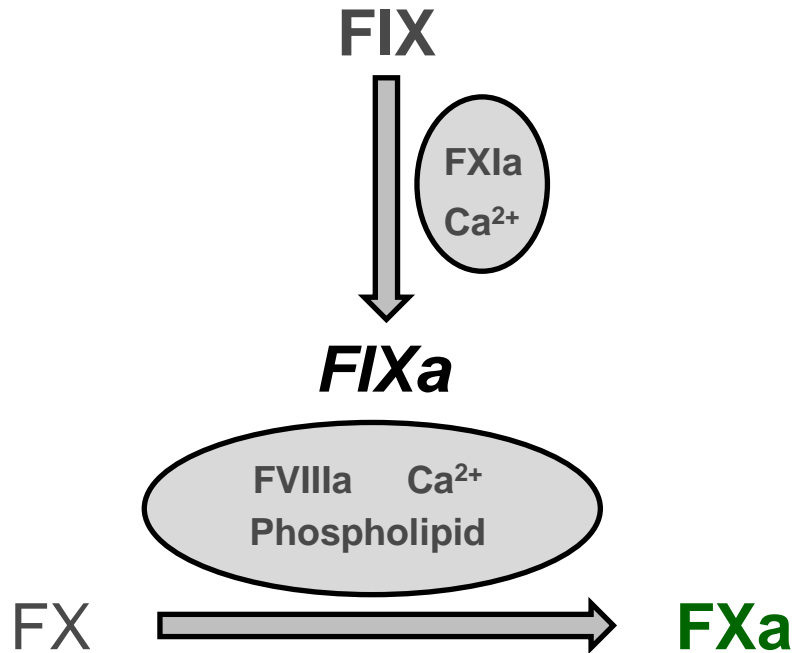


# Determination of FIX activity with Rox Factor IX

# Method Principle

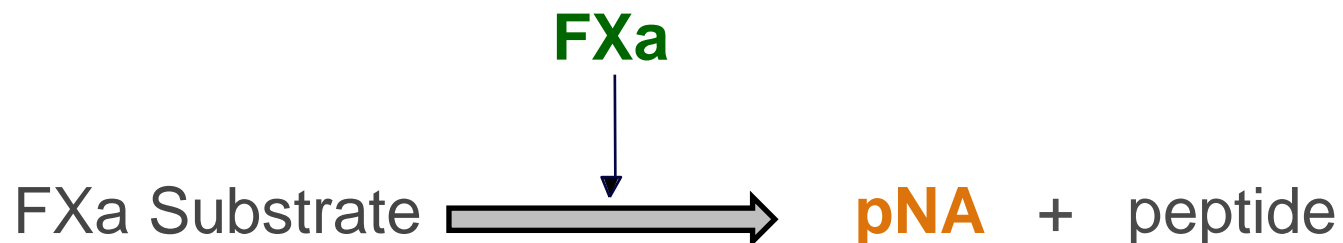
1



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



# Bioreagents in Rox Factor IX

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## **Reagent A**

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

## **Reagent B**

Human FXIa, human FII, calcium chloride and phospholipids.

### *Characteristics*

- Activation time **8 min**
- Max rate of FIX activation
- **FXa plateau** reached due to FVIIIa inactivation
- LOQ (limite of quantitation) 0.005 IU/mL

CE registration Feb 2014

# Manual Microplate End-Point Method

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Sample Dilution (18-25°C)	25 µL
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Reagent A (18-25°C)	25 µL
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*Preheating, 3-5 min at 37°C*

Reagent B (37°C)	150 µL
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*Activation, 8 min at 37°C*

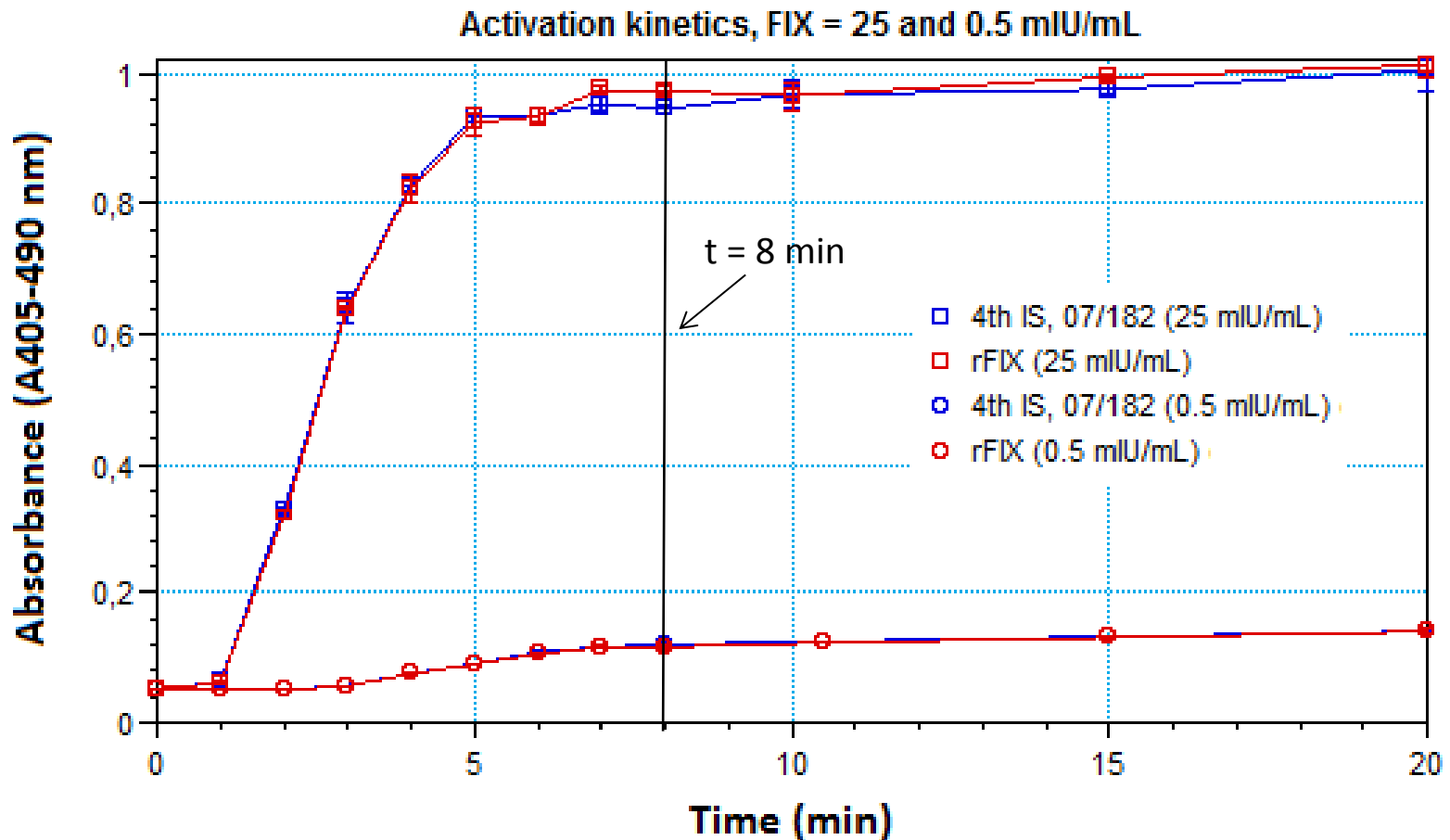
FXa Substrate (37°C)	50 µL
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*Hydrolysis, 2 min at 37°C*

Citric Acid, 2% (18-25°C)	50 µL
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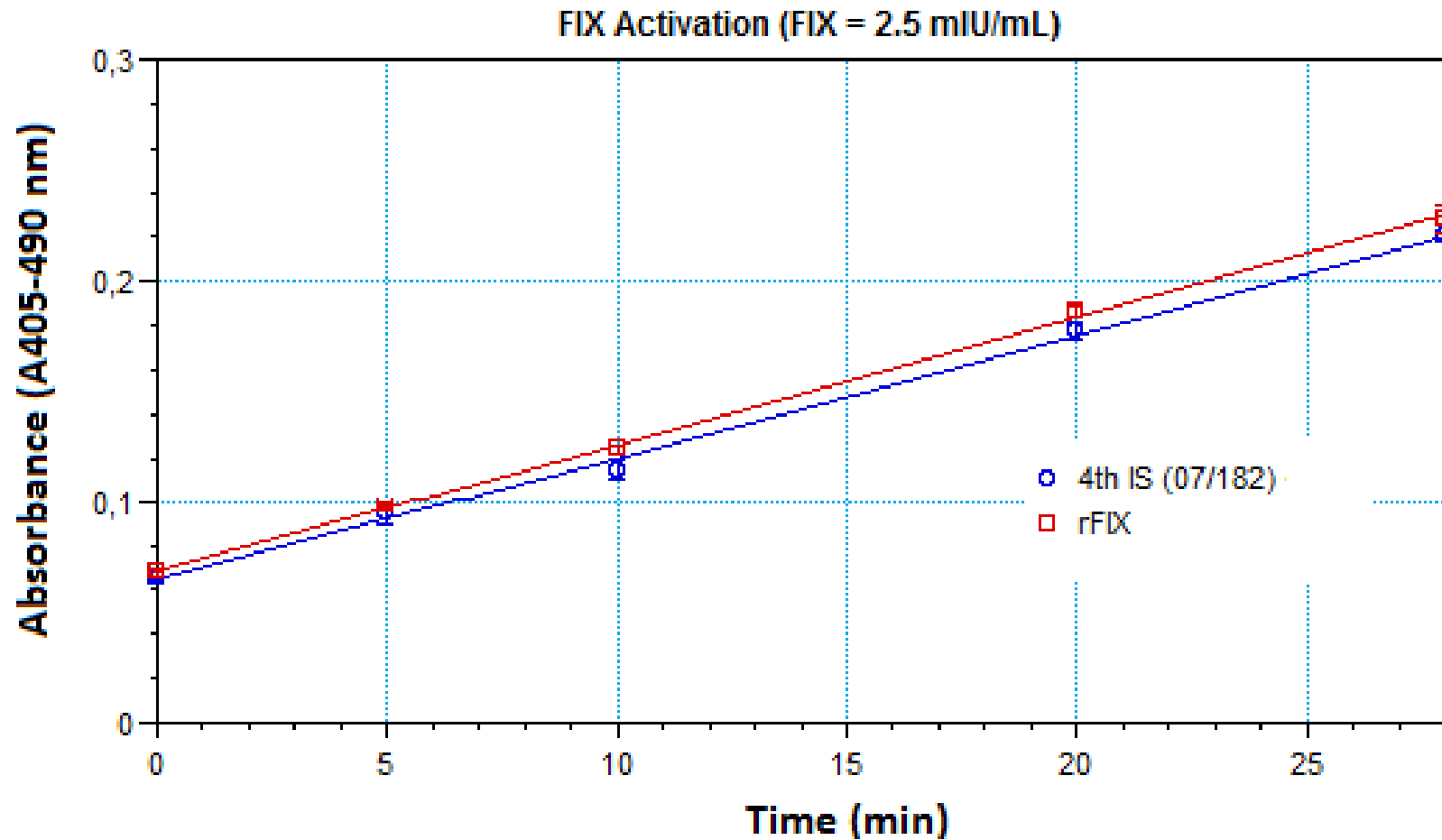
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# Method Principle Rox Factor IX, cont.



# Method Principle Rox Factor IX, cont.

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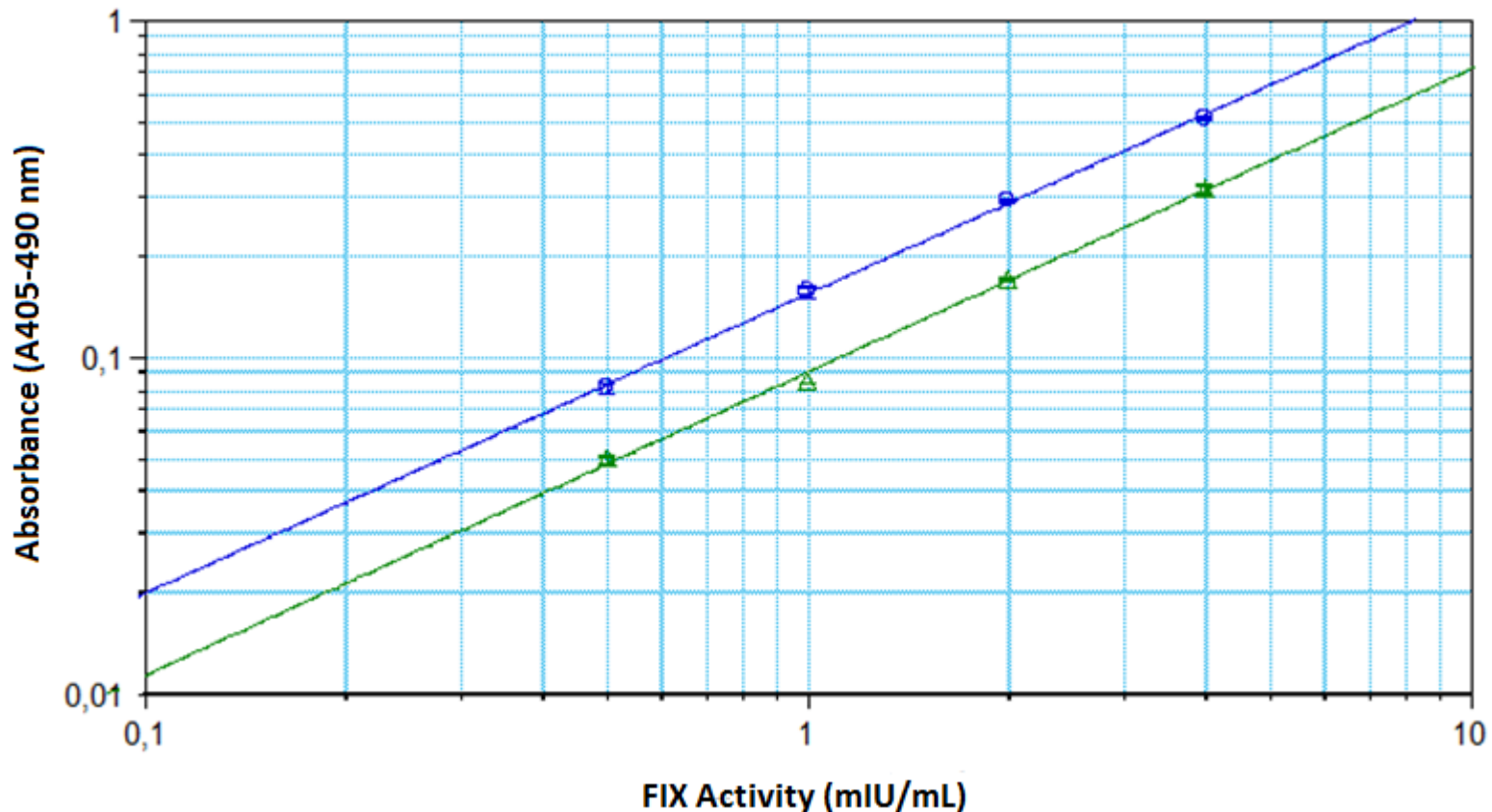
Mean assigned FIX potencies for rFIX and pdFIX vs 4th IS (07/182) at different activation times, using four dilutions of each sample.

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%)

r: recombinant and pd: plasma derived

# Parallel Line

Parallel line representation of a pdFIX concentrate sample (green) vs. the 4<sup>th</sup> IS FIX Concentrate (blue) in the range 0.5 - 4 mIU/mL, using 4 min hydrolysis at 37°C. The graph shows the mean (+ SD bars) results from simultaneous assay of two independently prepared dilution series of the 4<sup>th</sup> IS and the pdFIX sample.



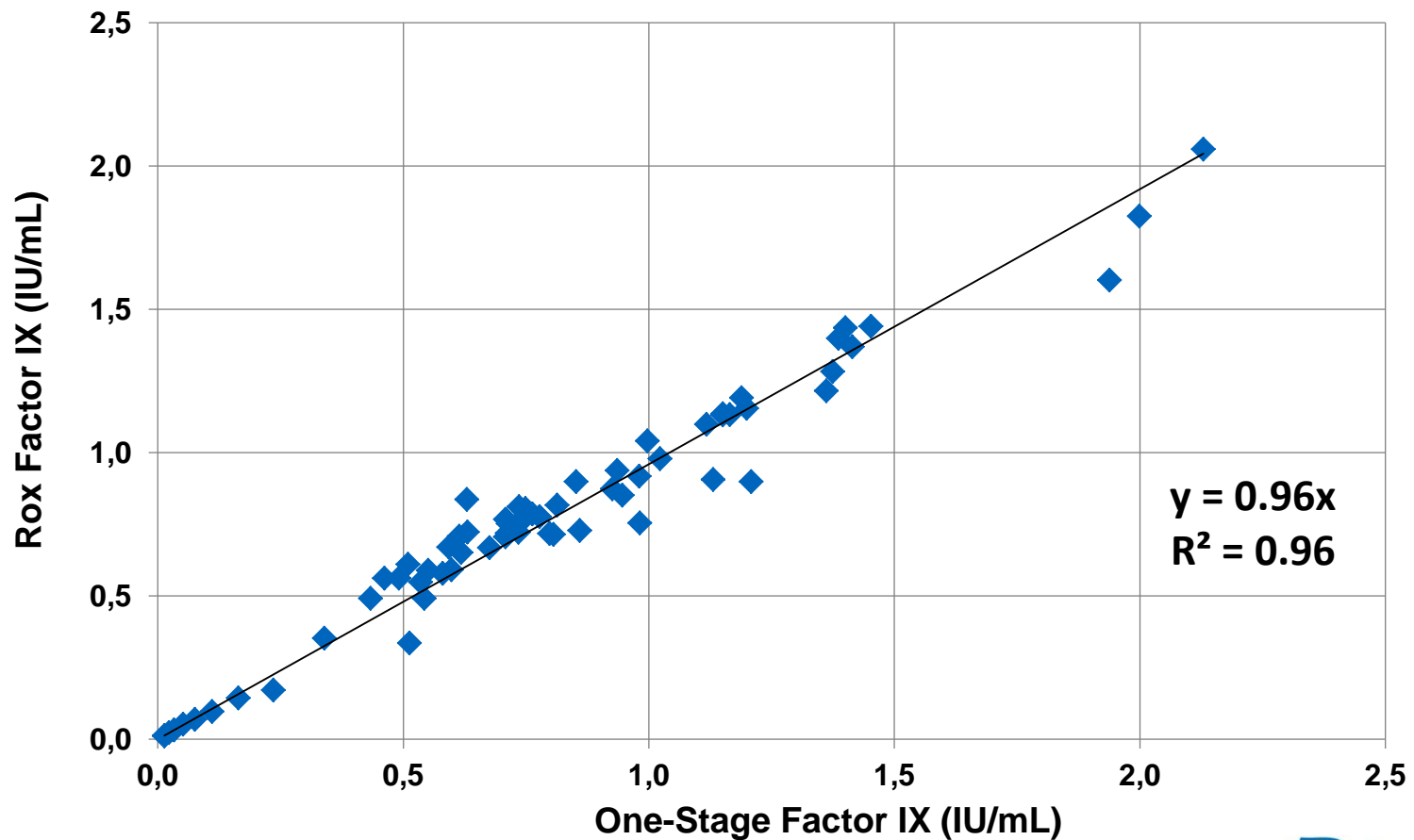
Note added Nov 11 2014: The pdFIX concentrate is Replenine



# Correlation to One-Stage Clotting method

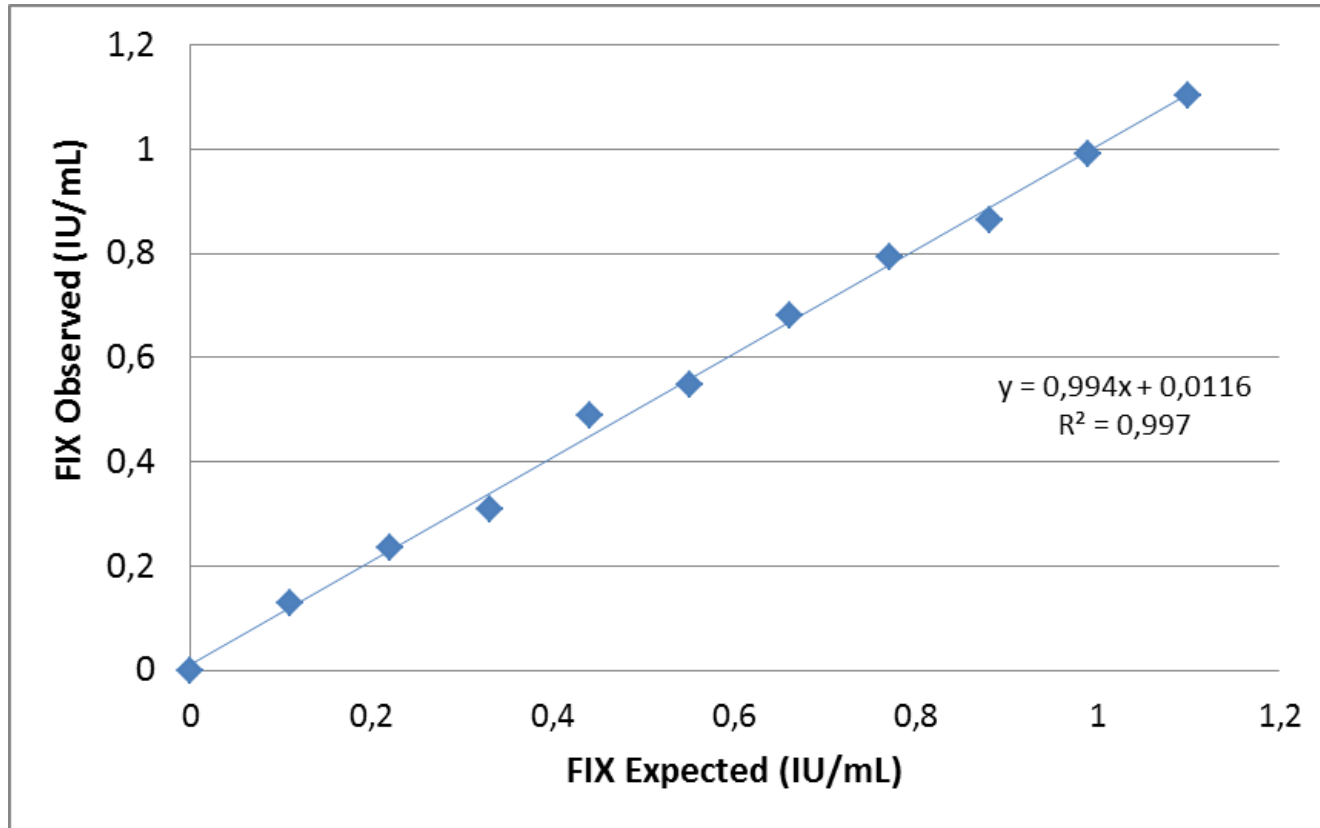
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Correlation to a One-Stage Clotting method comprising plasma from normals, hemophilia B patients and mixtures of FIX deficiency plasma and normal plasma (n=66)



# Dilutional Linearity, Rox Factor IX

Dilutional linearity obtained with step-wise dilution of one individual sample plasma with FIX deficient plasma analyzed on BCS XP at the clinical lab in Malmö



A proper linearity is obtained for all dilutions

# FIX determination in the absence and presence of FIX deficient plasma

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

Plasma Samples	Dilution in diluent	Standard curve	
		in diluent	in diluent
			+ 5% FIX def. plasma
10 mIU/mL	1/20	9.6 $\pm$ 0.1	9.2 $\pm$ 0.1
20 mIU/mL	1/20	20 $\pm$ 0.4	20 $\pm$ 0.4
53 mIU/mL	1/20	54 $\pm$ 0.9	53 $\pm$ 0.9
0.46 IU/mL	1/80	0.46 $\pm$ 0.01	0.46 $\pm$ 0.01

There is no difference between standard dilutions in buffer or in buffer + 5% deficient plasma FIX  $\rightarrow$  no plasma matrix effect.

# Activation kinetics – Rox Factor IX

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