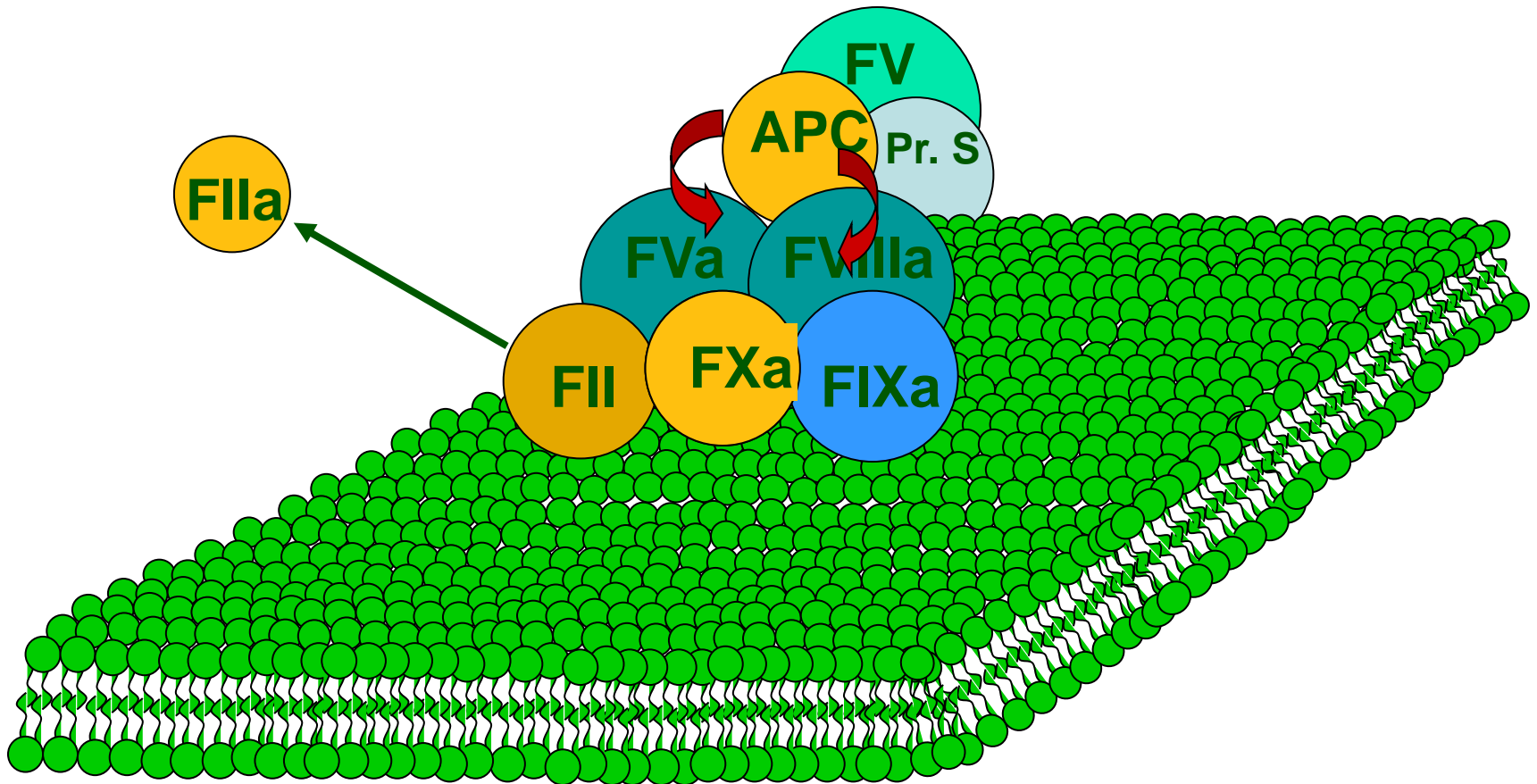


Rox Factor IX

For Research Use Only

Coagulation and its control

Formation and regulation of FXa and thrombin - a challenging system with heterogenous catalysis!



Bioreagents Rox Factor IX

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.

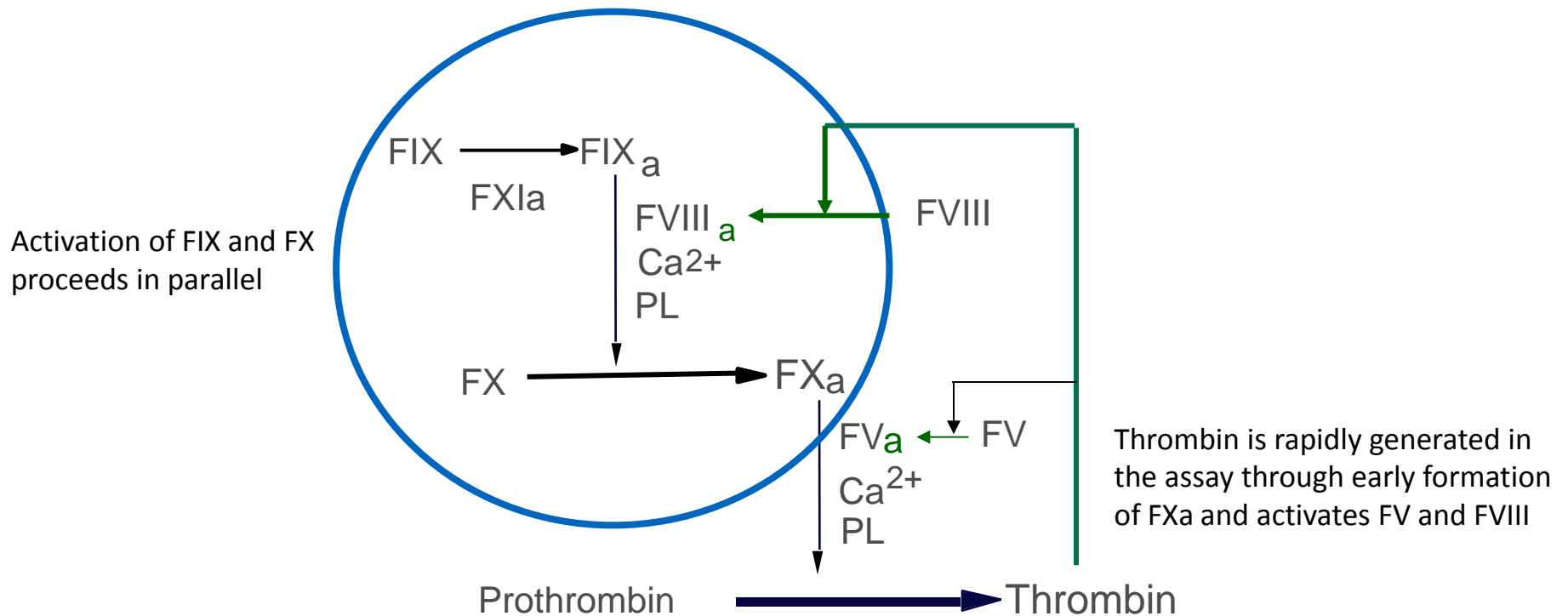
FIX Diluent Buffer, Stock Solution

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

No use of Factor IX deficient plasma

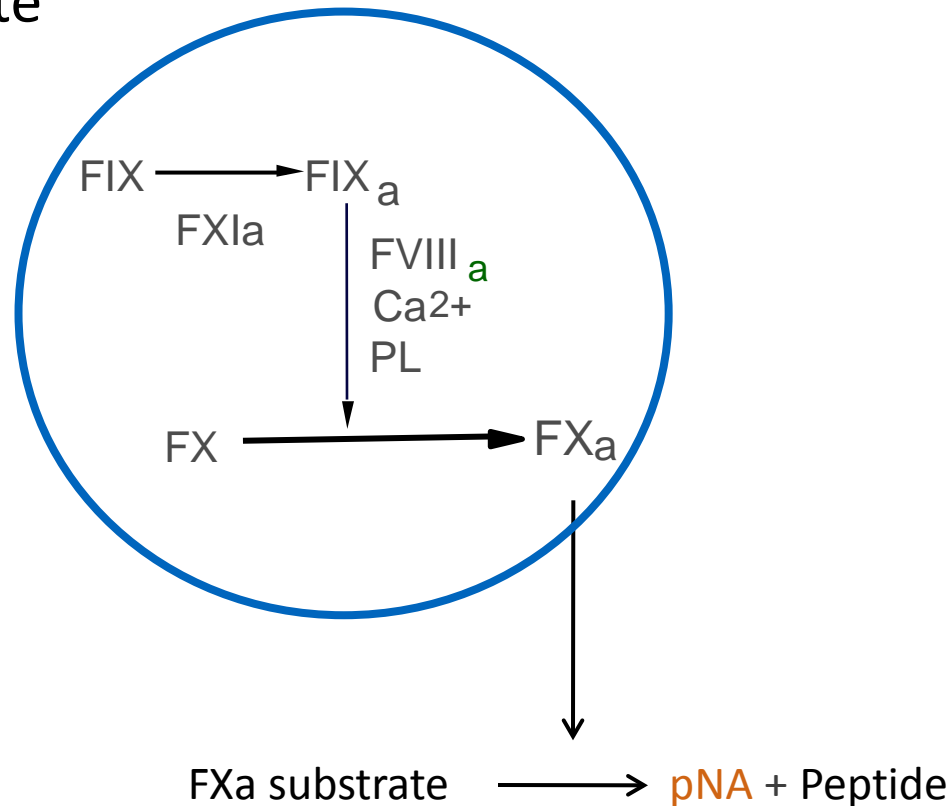
Approach used in two-stage chromogenic FIX method

1) FXa generation with FIX as rate limiting component



Approach used in two-stage chromogenic FVIII/IX methods

2) Determination of FXa from cleavage rate of a chromogenic FXa substrate



General aspects

- Neither one-stage nor chromogenic methods reflects physiological conditions, since there is no endothelial surface present in these in-vitro methods.
- One-stage clotting methods use non-physiological contact activators and the clotting times are much shorter than in vivo.
- Chromogenic methods use high sample dilutions resulting in considerably lower FVIII/FIX activities than in vivo.

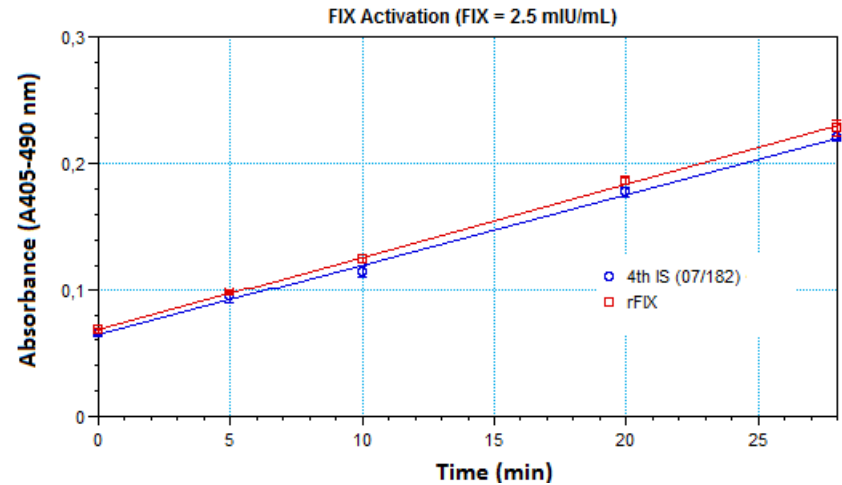
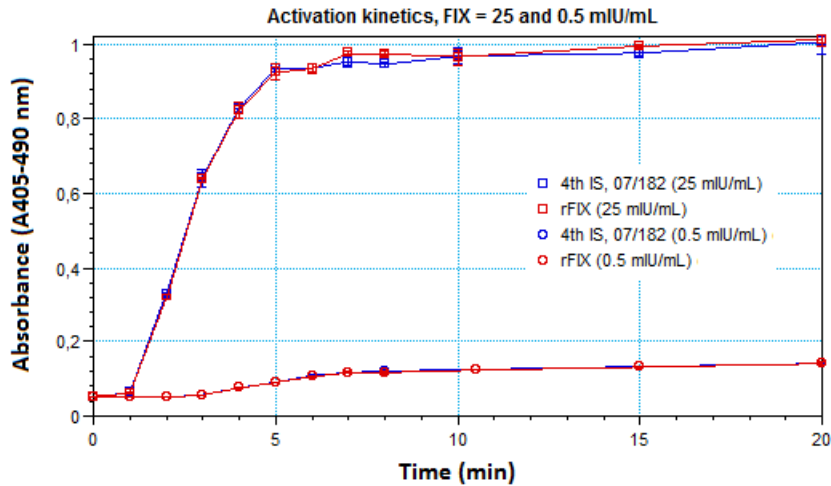
Chromogenic FIX methods, general

- Chromogenic methods involve relatively high sample dilutions and are generally less prone to interference than one-stage methods
- Chromogenic methods are not sensitive to preactivation of FVIII/FIX
- Activation times of chromogenic methods closer to physiological conditions as compared to one-stage methods

Chromogenic FIX methods, features (Rossix)

- Activation time 8 min
- Max rate of FIX activation
- FXa plateau reached due to FVIIIa inactivation, resulting in increased **assay robustness**
 - Any small variation in time and temperature of the instrumentation used will not affect the outcome
 - The plateau level of FXa is related to the FIX activity in the sample
- Activation of FIX starts immediately, while lag time for formation of FVIIIa is longer at very low FIX activities
 - **high sensitivity** - LOQ is 0.005 IU/mL (0.5%).

Method Principle Rox Factor IX, 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%)

r: recombinant and pd: plasma derived

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

LOQ of FIX activity is **0.005 IU/mL (0.5%)**

CE registration received in February 2014