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
1) FXa generation with FIX as rate limiting component

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

<table>
<thead>
<tr>
<th>Activation Time Min</th>
<th>rFIX IU/mL (CV%)</th>
<th>pdFIX IU/mL (CV%)</th>
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<tbody>
<tr>
<td>2.5</td>
<td>90 (2.2%)</td>
<td>85 (4.1%)</td>
</tr>
<tr>
<td>4</td>
<td>90 (3.6%)</td>
<td>88 (3.6%)</td>
</tr>
<tr>
<td>8</td>
<td>88 (2.0%)</td>
<td>87 (4.1%)</td>
</tr>
</tbody>
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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 ± FIX deficient plasma (n=4).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Standard in diluent</th>
<th>Standard in diluent + 5% FIX def. plasma</th>
</tr>
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<tbody>
<tr>
<td>10 mIU/mL</td>
<td>9.6 ± 0.1</td>
<td>9.2 ± 0.1</td>
</tr>
<tr>
<td>20 mIU/mL</td>
<td>20 ± 0.4</td>
<td>20 ± 0.4</td>
</tr>
<tr>
<td>53 mIU/mL</td>
<td>54 ± 0.9</td>
<td>53 ± 0.9</td>
</tr>
<tr>
<td>0.46 IU/mL</td>
<td>0.46 ± 0.01</td>
<td>0.46 ± 0.01</td>
</tr>
</tbody>
</table>

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

CE registration received in February 2014