COASET® FVII - 82 1900 63

Intended Use
For the photometric determination of factor VII activity in plasma such as when identifying elevated levels of Factor VII or factor VII deficiency and monitoring of patients on replacement therapy.

Principle
1. S-2765: FXa
2. FXa
3. CaCl₂
4. Thromboplastin

The method is based on a two-stage principle. In stage one Factor X is activated to FXa via the intrinsic pathway (FVII-thromboplastin). Factor VII is completely converted to FXa during this process and accordingly there is no interference in the assay of procoagulated FVII. In stage two the generated Factor Xa hydrolizes the chromogenic substrate S-2765 thus liberating the chromophoric group, pNA. The colour is then read photometrically at 405 nm. The generated Factor Xa and thus the intensity of colour is proportional to the FVII activity in the sample.

Composite
1. S-2765
2. Bovine Serum Albumin
3. Buffer stock solution
4. Factor X
5. CaCl₂
6. Thromboplastin

The Coasett FVII kit contains:
- 1 vial Chromogenic substrate (N-a-Cbo-D-Arg-Gly-Arg-pNA), (8 mg) with mannitol added (bulking agent).
- 1 vial Bovine Serum Albumin (BSA) 20%.
- 1 vial Buffer stock solution Tris 1.0 mol/L, pH 7.4.
- 1 vial Factor X
- 1 vial Calcium chloride solution, 40 mmol/L.
- 1 vial Thromboplastin

PRECAUTION AND WARNING
Each donor unit used in the preparation of human source reagent has been tested by FDA approved methods for the presence of Hepatitis B surface antigen and antibodies to HIV 1 and 2 and Hepatitis C and found to be negative. However, since no test can completely rule out the presence of these blood borne diseases, the handling and disposal of all source reagents from this product should be made with care. Handle as potentially infectious13.

Harritel it swallowed (R22). Avoid contact with skin and eyes (S24/25). Do not empty as potentially infectious15.

Disposal of human source reagents from this product should be made with care. Handle as potentially infectious15.

The opened vial is stable for 1 week at 2-8°C. The sealed reagents are stable until the expiry date printed on the label.

COASET® FVII is Ready to use label. Contamination by microorganisms should be avoided once the vials are opened.

5. CaCl₂: Stable at 2-8°C until the expiry date printed on the label.
6. Thromboplastin: Reconstituted thromboplastin is stable for 1 month at 2-8°C.

Reagents not provided:
- Normal human plasma for calibration, which should be calibrated against an International Standard. Three levels of FVII activity are provided. Each level should be stored at 2-8°C. For optimal results, store at 2-8°C or for three months at -20°C. Avoid freezing. Thaw rapidly at 37°C just before use.
- Acetic acid 20% or citric acid 2%.
- Sterile water11

Materials required but not provided:
- Photometer, 405 nm (and 490 nm for the microplate procedure)
- Heat incubator 37°C ±0.2°C. Verify that the incubator provides an even temperature distribution within these specifications.
- Calibrated pipettes with an accuracy of 1% or better.
- Plastic test tubes
- Semi-micro cuvettes
- Vortex mixer
- Sample dilute/dispense block
- Stopwatch
- Centrifuge, 2000g

specimen collection and Preparation
Blood (9 volumes) is mixed with 0.1mol/L sodium citrate (1 volume). The first 3-5 mL of blood is discarded. Centrifuge at 2000g for 20 minutes. Separate the plasma from the cells within two hours of collection. If the plasma is not used immediately, store at 2-8°C or at room temperature for a maximum of 4 hours. The plasma may also be dispensed in aliquots and kept frozen at -70°C for a maximum of three months before testing. Avoid refreezing. Thaw rapidly at 37°C just before use.

Quality Control
Two levels of FVII controls, calibrated against International Standards, are recommended for a complete quality control program. Each laboratory should establish its own mean and standard deviation and should establish a quality program to monitor laboratory testing. Controls should be analyzed at least once every 8 hour shift in accordance with laboratory procedure. Refer to Westgard et al for identification and resolution for out-of-control situations.

Procedure
a) Prepare a Combined reagent by mixing:
1 part Thromboplastin dilution
5 parts FVII 0.2 IU/mL
3 parts CaCl₂, 40 mmol/L
The mixture is stable for 30 minutes at 37°C or 3 hours at 2-8°C.
b) Preparation of Calibrators.
A Calibration curve is required for each series of assays. Normal human plasma, calibrated against an International Standard, is used for standardization. This plasma is diluted in Tris-BSA working buffer accordingly:


<table>
<thead>
<tr>
<th>%FVII</th>
<th>Dilution with Tris-BSA working buffer</th>
<th>%FVII</th>
<th>Dilution with Tris-BSA working buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.100</td>
<td>1.000</td>
<td>0.100</td>
<td>1.000</td>
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<tr>
<td>0.050</td>
<td>1.000</td>
<td>0.050</td>
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<tr>
<td>0.025</td>
<td>1.000</td>
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<td>1.000</td>
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<tr>
<td>0.0125</td>
<td>1.000</td>
<td>0.0125</td>
<td>1.000</td>
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</tbody>
</table>

2) Identical FVII activities were obtained with normal plasma prediluted 2-8 times in either buffer or FVII deficiency plasma before the final dilution of 1:1000 in buffer. A 1:2 fold predilution of a plasma sample containing a high FVII concentration (pregnant woman, 3rd trimester) in FVII deficiency plasma compared to predilution in buffer resulted in assigned values of 236% and 229% respectively after correction for the dilution.

Heparin does not interfere at levels ≤ 0.5 IU/mL plasma.

Precision

<table>
<thead>
<tr>
<th>Microplate</th>
<th>CV% (within run)</th>
<th>n</th>
<th>CV% (Total)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (% FVII)</td>
<td>119</td>
<td>3.5</td>
<td>5</td>
<td>5.6</td>
</tr>
<tr>
<td>35</td>
<td>3.5</td>
<td>5</td>
<td>4.9</td>
<td>35</td>
</tr>
</tbody>
</table>

Correlation

<table>
<thead>
<tr>
<th>System slope</th>
<th>intercept</th>
<th>Reference Method n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microplate</td>
<td>0.97</td>
<td>11.2</td>
</tr>
</tbody>
</table>

A comparison to an ELISA antigen assay was also performed for samples from normal healthy individuals (n=27) with average values of (±2SD) = ±0.102 (52%) and 102 (22%) for Coasett FVII and the ELISA method respectively. As expected, higher values were obtained for the ELISA method (±11.4%) as compared to Coasett FVII (±8.1%) when plasma from patients on oral anticoagulant therapy (n=42) were assayed.
The assay can also be conveniently performed in microplates and will then permit 120 determinations. Care should be taken to keep to the reaction conditions as described above; however, when using proportional volume changes, e.g. from 200 ml to 50 ml, the separate reaction time should be prolonged from 5 to 7 minutes. Start a timer when the combined reagent is added so that each sample is activated for 7 minutes. Then add the S-2765 at the same intervals as for the combined reagent. Use the same procedure for acetic acid or citric acid after 7 minutes to stop the reaction. Dual wavelength mode reading is preferable to compensate for differences between the substrate reaction time should be prolonged from 5 to 7 minutes. Start a timer when the combined reagent is added so that each sample is activated for 7 minutes. Then add the S-2765 at the same intervals as for the combined reagent. Use the same procedure for acetic acid or citric acid after 7 minutes to stop the reaction. Dual wavelength mode reading is preferable to compensate for differences between the microplate wells. 

**Alternative Procedure**

The assay can also be conveniently performed in microplates and will then permit 120 determinations. Care should be taken to keep to the reaction conditions as described above; however, when using proportional volume changes, e.g. from 200 ml to 50 ml, the separate reaction time should be prolonged from 5 to 7 minutes. Start a timer when the combined reagent is added so that each sample is activated for 7 minutes. Then add the S-2765 at the same intervals as for the combined reagent. Use the same procedure for acetic acid or citric acid after 7 minutes to stop the reaction. Dual wavelength mode reading is preferable to compensate for differences between the microplate wells. 

**Symbols used**

- **IVD**: In-vitro diagnostic device
- **LOT**: batch code
- **IVD** system
- **LO**
- **EC**: Authorised representative
- **EC**: Bevollmächtigter
- **REP**: Representante autorizado
- **REP**: Representante autorizado
- **REP**: Autoriserad representant

**Bibliography**

15. RICHARDSON J H and BACKLEY W E. Eds. Biosafety in Microbiological and Biomedical Laboratories. US. Dept of Health and Human Services, Public Health Service, HMS