BIOPHEN FVII
Ref 221304
Assay for measuring Factor VII in plasma with a chromogenic method
In vitro research use only

INTENDED USE:
BIOPHEN FVII kit is a chromogenic assay proposed for measuring the Factor VII activity in human plasma or in biological fluids, using a chromogenic method, manual or automated.

CLINICAL APPLICATIONS:
Diagnosis of congenital or acquired Factor VII deficiencies. Assay of Factor VII activity, in citrated human plasma or in any biological fluid where Factor VII activity must be measured.

ASSAY PRINCIPLE:
Factor VII is the serine esterase of the extrinsic coagulation pathway. When complexed to Tissue Factor (TF), in presence of phospholipids and Calcium, it activates Factor X to Factor Xa. Biophen Factor VII is a chromogenic assay for testing Factor VII activity. Factor VII forms an enzymatic complex with Tissue Factor, provided by rabbit Thromboplastin. It then activates Factor X, present in the assay at a constant concentration and in excess, to Factor Xa, which concentration is exactly measured by its activity on a specific Factor Xa chromogenic substrate (SXa-11). Factor Xa cleaves the substrate and generates pNA. The amount of pNA generated is directly proportional to the Factor Xa activity. Finally, there is a direct relationship between the amount of Factor VII in the assayed sample and the concentration is exactly measured by its activity on a specific Factor Xa chromogenic substrate (SXa-11).

REAGENTS:
R1: Reagent 1: Human Factor X
Human Factor X, lyophilised:
2 vials containing Factor X, at the optimized concentration for the assay (to be reconstituted with 4 mL of distilled water).

R2: Reagent 2: Calcium-Thromboplastin
Rabbit brain thromboplastin, containing calcium, lyophilised:
2 vials containing 8 mg of calcium-Thromboplastin, containing calcium (to be reconstituted with 2 mL of distilled water).

R3: Reagent 3: SXa-11
Chromogenic substrate, specific for Factor Xa (SXa-11), lyophilised:
2 vials containing 8 mg of SXa-11 (to be reconstituted with 4 mL of distilled water).

R4: Reagent 4: Tris-BSA Buffer
Ready to use buffer, at pH 7.40, contains sodium azide (NaN3), 4 vials of 25 mL.

Warning:
• Human Factor X was prepared from human plasma, which was tested with registered methods and found negative for HIV antibodies, Hbs Ag and HCV antibodies. However, no assay may warrant the total absence of infectious agents. Any product of human origin must then be handled with all the required cautions, as being potentially infectious.

PREPARATION OF PLASMA:
Blood (9 volumes) must be collected on 0.109 M citrate anticoagulant (1 volume), with great care, in a silicon glass or a plastic tube. Sampling must be performed through a net venipuncture, avoiding any contamination.

Within 4 hours, blood must be centrifuged at 3,000 g for 20 min at 18°C or below, and plasma decanted into a plastic tube, using a plastic pipette.

The stability studies at 30°C show that the reagents can be shipped at room temperature without damage.

CAUTIONS:
• In order to improve stability, reagents must be closed with their original screw cap following each use (white caps for factor X (R1), Calcium-Thromboplastin (R2) and buffer (R4), yellow caps for SXa-11 (R3)).

PREPARATION AND STABILITY OF REAGENTS:
R1: Reagent 1: Human Factor X
- Reconstitute each vial with exactly 4 mL of distilled water.
- Leave to homogenize for 30 minutes at room temperature (18-25°C).
- Shake gently before use.

Stability of reconstituted human Factor X, kept in its original vial:
• 48 hours at 2-8°C.
• 8 hours at Room Temperature (18-25°C).
• Do not freeze.

R2: Reagent 2: Calcium-Thromboplastin
- Reconstitute each vial with 2 mL of distilled water.
- Leave to homogenize for 30 minutes at room temperature (18-25°C).
- Shake gently before use.

Stability of restored reagent, kept in its original vial:
• 48 hours at 2-8°C.
• 8 hours at Room Temperature (18-25°C).
• Do not freeze.

R3: Reagent 3: Factor Xa specific Chromogenic substrate (SXa-11)
- Reconstitute each vial with 4 mL of distilled water.
- Incubate at Room Temperature (18-25°C) for 30 minutes.
- Shake gently before use.

Stability of restored substrate, kept in its original vial:
• 3 months at 2-8°C.
• 7 days at Room Temperature (18-25°C).
• Do not freeze.

R4: Reagent 4: Tris-BSA Buffer
Ready to use buffer, vial of 25 mL. Shake before use. It contains Sodium Azide (0.9 g/L).

This reagent is stable until the expiration date printed on the label, when stored at 2-8°C, protected from any contamination.

Material:
• Distilled water, preferably sterile.
• Acetic Acid (20%) or Citric Acid (2%) (End point method).

TEST PROCEDURE:
BIOPHEN FVII kit is designed for use with automated kinetic methods but it can also be used for end point manual methods. Adaptations for the various automates are available upon request. The assay is performed at the controlled temperature of 37°C and the colour development is measured at 405 nm.
CALIBRATION:

Calibration is performed with a normal pooled citrated plasma (at least 30 normal individuals, males or females, aged between 18 and 55 years, and free of any medication or disease), with the assigned value of 100 % Factor VII. The assay includes a standard plasma dilution of 1:1,000. By definition, this latter dilution of the pool represents the 100 % Factor VII activity. The dynamic range is from 0 to 200 % Factor VII. The 200 % Factor VII activity is the 1:500 dilution of the plasma pool.

In order to have an accurate dilution, predilute the pool plasma at 1:25, then 1:20 (i.e. 1:500 final dilution) with Tris-BSA buffer (R4). Using this dilution, prepare the calibration range as indicated here below:

<table>
<thead>
<tr>
<th>% FVII</th>
<th>Plasma Calibrator (µL) Diluted 1/500</th>
<th>Tris-BSA Buffer (R4) (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>50</td>
<td>125</td>
<td>375</td>
</tr>
<tr>
<td>100</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>200</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

ASSAY PROTOCOL:

Manual Method:
Tested plasmas and controls are assayed at the 1:500 dilution in Tris-BSA buffer (R4).

For therapeutic concentrates or for biological fluids with Factor VII concentrations different from that of plasma, dilute the sample in order to get a final Factor VII concentration in the tested dilution in the range 0.1 to 1 ng/mL (i.e. 20 to 200 % Factor VII, using this protocol).

Automated methods:
Adaptations for the various analysers are available upon request. The assay is then performed kinetically. The reaction does not require to be stopped and sample blanks are automatically subtracted.

RESULTS:
For the end-point method, using a bilogarithmic scale, plot on abscissa the Factor VII concentration (%) and on ordinates the corresponding absorbance (A405).

The factor VII concentration in the tested sample is directly obtained on the calibration curve. Results are expressed as % of a normal plasma pool.

- Using automated methods, the Factor VII concentrations are directly calculated by the analyser, respectively to the calibration curve, and the sample dilution used.
- The dynamic range is from 5 to 200 %.

When the assay dilution is 1:1,000, the Factor VII concentration is directly read on the calibration curve. When different dilutions are used, the results must be multiplied by the dilution factor “D”, divided by 1,000, i.e. D/1,000.

PERFORMANCE CHARACTERISTICS:

The detection threshold for the assay is evaluated on the calibration curve by measuring the "apparent" Factor VII concentration, which corresponds to the mean A405 value obtained for a sample free of Factor VII plus 3 Standard Deviations (SD). This detection threshold is of 5 % for the Biophen Factor VII kit.

REFERENCES: