COASET® FVII - 82 1900 63

ENGLISH - Insert revision 09/2003

CE

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. FX $\xrightarrow[\text{FVII}]{\text{Thromboplastin, } Ca^{2+}}$ FXa

2. S-2765 $\xrightarrow{\text{FXa}}$ Peptide + pNA (yellow colour)

The method is based on a two-stage principle. In stage one Factor X is activated to FXa via the extrinsic pathway (FVII-thromboplastin). Factor VII is completely converted to FVIIa during this process and accordingly there is no interference in the assay of preactivated FVII. In stage two the generated Factor Xa hydrolyses 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.

Composition

The Coaset FVII kit contains:

1. **S-2765** — 1 vial
   Chromogenic substrate (N-a-Cbo-D-Arg-Gly-Arg-pNA), (8 mg) with mannitol added (bulking agent).
2. **Bovine Serum Albumin** — 1 vial
   BSA 20%
3. **Buffer stock solution** — 1 vial
   Tris 1.0 mol/L, pH 7.4
4. **Factor X** — 1vial
   Bovine Factor X, 3.1 IU.
5. **$CaCl_2$** — 1 vial
   Calcium chloride solution, 40 mmol/L.
6. **Thromboplastin** — 1 vial
   Human placenta 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 human source reagents from this product should be made with care. Handle as potentially infectious[15].
Harmful if swallowed (R22). Avoid contact with skin and eyes (S24/25). Do not empty into drains (S29). Wear suitable protective clothing (S36).

This product is for *in vitro* diagnostic use.

Preparation

1. **S-2765:** Reconstitute with 6 mL of sterile water[11] to obtain a concentration of 1.87 mmol/L..
2. **Bovine Serum Albumin**: Ready to use
3. **Buffer stock solution:** Before use dilute accordingly:
   1) 1 volume of stock solution with 19 volumes of sterile water[11].
   2) To 100 volumes of this solution add 1 volume of BSA 20%. (Tris-BSA working buffer containing 0.2% BSA)
4. **Factor X:** Reconstitute in 1.0 mL sterile water[11]. Before use, dilute further by mixing 1 volume of Factor X with 3 volumes of Tris-BSA working buffer to obtain a concentration of 0.8 IU/mL.
5. **$CaCl_2$:** ready to use
6. **Thromboplastin:** Reconstitute with 4.0 mL of sterile water[11]. Before use, dilute further by mixing 1 volume of Thromboplastin with 4 volumes of Tris-BSA working buffer.

Reagent storage and Stability

When kept at 2-8°C the sealed reagents are stable until the expiry date printed on the label. Contamination by microorganisms should be avoided once the vials are opened.

1. **S-2765:** Reconstituted substrate is stable for 6 months at 2-8°C.
2. **Bovine Serum Albumin:** The opened vial is stable for 1 week at 2-8°C.
3. **Buffer stock solution:** The stock solution is stable for 2 months at 2-8°C. Tris-BSA working buffer is stable for 5 days at 2-8°C.
4. **Factor X:** Reconstituted Factor X is stable for one week at 2-8°C.
5. **$CaCl_2$:** 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. Blood samples are taken from at least 20 healthy donors. 10-30 mL of citrated blood (9 volumes blood and 1 volume 0.1 mol/L sodium citrate) are taken from each donor. The first 3-5 mL of blood is discarded. Plasma is prepared by centrifugation at 2000xg for 20 minutes. Equal amounts of plasma from the donors are mixed and dispensed in small volumes and frozen immediately. The normal plasma is stable for one year at -70°C or for three months at -20°C. Avoid refreezing. Thaw rapidly at 37°C just before use.

— Acetic acid 20% or citric acid 2%.

— Sterile water[11]

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 diluter/dispenser

— Stopwatch

— Centrifuge, 2000xg

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 2000xg for 20 minutes.
Separate the plasma from the cells within two hours of collection. If the plasma is not tested 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.[13] 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 good laboratory practice. Refer to Westgard et al for identification and resolution for out-of-control situations.[14]

Procedure

a) Prepare a Combined reagent by mixing:

1 part	Thromboplastin dilution
5 parts	FX 0.8 IU/mL
3 parts	$CaCl_2$ 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:

%FVII	Dilution with Tris-BSA working buffer
200	1:500
100	1:1000
50	1:2000
25	1:4000
12.5	1:8000

c) Preparation of plasma sample.

The plasma sample is diluted 1:1000 in Tris-BSA working buffer. The plasma dilutions are stable for at least 2 hours at 2-8°C. For optimal results, high precision diluting equipment is recommended.

d) Assay

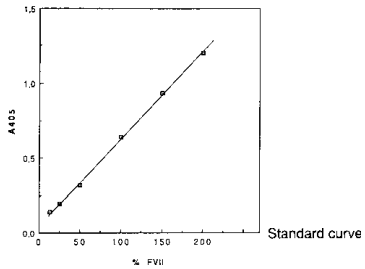
A reagent blank (Tris-BSA working buffer instead of sample) should be included in each series. Place the required volume of substrate and Combined reagent at 37°C.

Add to a plastic test tube:	Volumes
Standard, sample dilution or Tris-BSA working buffer (2-8°C)	200 µL
Incubate 3-4 minutes at 37°C	
Combined reagent (37°C)	200 µL
Mix and incubate 7 minutes at 37°C	
S-2765 (37°C)	200 µL
Mix and incubate 5 minutes at 37°C	
Acetic Acid, 20% or citric acid 2%	200 µL

Read the absorbance in a photometer at 405 nm against the reagent blank. The colour is stable for at least 4 hours.

Calculation

Plot the absorbance (A405) for the standards against their concentration of FVII on a linear graph paper. Read the %FVII value for the corresponding absorbance for the unknown sample from the standard curve.



Results

FVII results are reported in activity (%).

Expected values

Males 54-138 (2 SD, n = 31), Females 56 – 200 (2 SD, n = 30) [12].

Performance Characteristics

Specificity and Interfering Factors

1) Addition of rabbit anti-human FVII IgG blocked >98% of the amidolytic activity.
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.
3) A six-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

Microplate	CV% (within run)	n	CV% (Total)	n
Mean (% FVII)				
119	3.5	5	5.6	35
35	3.5	5	4.9	35

Correlation

System	slope	intercept	r	Reference Method	n
Microplate	0.97	11.2	0.96	FVII Recombiplastin (ACL Futura)	47

A comparison to an ELISA antigen assay was also performed for samples from normal healthy individual (n=27) with average values of (±+2SD) =108±25% and 102 ±22% for Coaset FVII and the ELISA method respectively. As expected, higher values were obtained for the ELISA method (51±14%) as compared to Coaset FVII (38 ±19%) when plasmas from patients on oral anticoagulant therapy (n=42) were assayed.

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Linearity
System

Microplate	12.5 - 200 % FVII

Sensitivity
System

Microplate : 6.7 ΔmAbs /min per 1% of FVII activity.

Detection Limit
System

Microplate: 3% FVII.

Alternative Procedure

The assay can also conveniently be 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 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. ΔA(405-490nm) should preferably be ≤1.1 absorbance units in order to avoid non linearity due to substrate depletion. It is of crucial importance to use microplates which have low adsorptivity of proteins. Thus, microplates intended for immunoassays should be avoided. Also verify that the microplate incubator provides an even temperature distribution within the specifications above.

Bibliography / Literatur / Bibliografía / Bibliographie / Bibliografia /Bibliografia / Litteratur / Litteraturförteckning / Βιβλιογραφία

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Symbols used / Verwendete Symbole / Símbolos utilizados / Symboles utilisés / Simboli impiegati / Símbolos utilizados / Anvendte symboler / Använda Symboler / Χρησιμοποιηθέντα σύμβολα

IVD	LOT				CONTROL			EC REP
In vitro diagnostic medical device	Batch code	Use by	Temperature limitation	Consult instructions for use	Control	Biological risks	Manufacturer	Authorised representative
In-vitro Diagnostikum	Chargen-Bezeichnung	Verwendbar bis	Festgelegte Temperatur	Beilage beachten	Kontrollen	Biologisches Risiko	Hergestellt von	Bevollmächtigter
De uso diagnóstico *in vitro*	Identificación número de lote	Caducidad	Temperatura de Almacenamiento	Consultar la metódica	Control	Riesgo biológico	Fabricado por	Representante autorizado
Dispositif mèdical de diagnostic *in vitro*	Désignation du lot	Utilisable jusqu'à	Températures limites de conservation	Lire le mode d'emploi	Contrôle	Risque biologique	Fabricant	Mandataire
Per uso diagnostico *in vitro*	Numero del lotto	Da utilizzare prima del	Limiti di temperatura	Vedere istruzioni per l'uso	Controllo	Rischio biologico	Prodotto da	Rappresentanza autorizzata
Dispositivo médico para utilização em diagnóstico *in vitro*	Número de lote	Data límite de utilização	Límite de temperatura	Consultar as instruções de utilização	Controlo	Risco biológico	Fabricado por	Representante autorizado
"in vitro" diagnostisk udstyr	Batch nr.	Anvendelse	Temperatur begrænsninger	Se vejledning for anvendelse	Kontrol	Miljø oplysninger	Producent	Leverandør
In vitro diagnostisk medicinsk produkt	Tillverkningskod	Användning	Temperatur gräns	Ta del av instruktionen före användning	Kontroll	Biologiska risker	Tillverkare	Auktoriserad representant
Προϊόν για διαγνωστική χρήση *In vitro*	Αρ. Παρτίδας	Χρήση έως	Περιορισμοί θερμοκρασίας	Συμβουλευτήτε τις οδηγίες χρήσης	Υλικό ποιοτικού ελέγχου	Βιολογικοί κίνδυνοι	Κατασκευαστής	Εξουσιοδοτημένος αντιπρόσωπος

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