COATEST® SP FVIII - 82 4086 63

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Intended use

For the photometric determination of factor VIII activity in citrated plasma, such as when identifying factor VIII deficiency or monitoring patients on replacement therapy, as well as for potency estimation of FVIII concentrates.

Measurement principle

$$\text{1. Factor X} \xrightarrow[\text{factor VIII}]{\text{Factor IXa, } Ca^{2+}\text{, phospholipid}} \text{factor Xa}$$

$$\text{2. S-2765} \xrightarrow{\text{factor Xa}} \text{Peptide + pNA}$$

In the presence of calcium and phospholipids, factor X is activated to factor Xa by factor IXa. This generation is greatly stimulated by factor VIII, which may be considered as a cofactor in this reaction. By using optimal amounts of Ca^{2+} and phospholipids and an excess of factors IXa and X, the rate of activation of factor X is solely dependent on the amount of factor VIII. Factor Xa hydrolyses the chromogenic substrate S-2765 thus liberating the chromophoric group, pNA. The color is then read photometrically at 405 nm. The generated factor Xa and thus the intensity of color is proportional to the factor VIII activity in the sample. Hydrolysis of S-2765 by thrombin formed is prevented by the addition of the synthetic thrombin inhibitor, I-2581, together with the substrate.

Composition

1. **S-2765 15.4 mg + I-2581 1 vial**
   Chromogenic substrate (N-a-Z-D-Arg-Gly-Arg-pNA), 15.4 mg, synthetic thrombin inhibitor, 0.4 mg, and mannitol added as a bulking agent.
2. **Factor IXa + factor X 9.2 IU 1 vial**
   Lyophilized bovine factors IXa and X with bovine albumin added as a stabilizing agent.
3. **CaCl2 6 ml 1 vial**
   Calcium chloride solution, 0.025 mol/L
4. **Buffer, stock solution 20 mL 1 vial**
   20 mL concentrated Tris buffer containing NaCl and BSA. Characteristics of tenfold diluted buffer: Tris 0.05 mol/L, pH 7.3, 10 mg/L Ciprofloxacin and 1.0% BSA.
5. **Phospholipid 2 mL 1 vial**
   Mixture of highly purified phospholipids and 10 mg/L Ciprofloxacin.

PRECAUTION AND WARNINGS
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

The reagents are reconstituted according to the specific instrument application. For microplate and test tube techniques:

1. **S-2765 + I-2581:** Reconstitute with 12.0 mL of sterile water or NCCLS type II water[11], to obtain a concentration of 2.7 mmol/L.
2. **Factor IXa + Factor X:** Reconstitute with 10.0 mL of sterile water or NCCLS type II water[11]
3. **$CaCl_2$:** Ready to use.
4. **Buffer, stock solution:** Dilute 1:10 (1+9) with sterile water or NCCLS type II water[11]. Prepare a new buffer working solution each day.
5. **Phospholipid:** Ready to use.

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 + I-2581:** Stability after reconstitution: 3 months at 2-8°C.
2. **Factor IXa + Factor X:** Stability after reconstitution: 12 hours at 2-8°C. The solution can be stored frozen in aliquots at -20°C (or at lower temperature) for 3 months. Do not refreeze.
3. **$CaCl_2$ (0.025 mol/L):** Opened vial is stable 3 months at 2-8°C.
4. **Buffer, stock solution (Tris 0.05 mol/L, pH 7.3, 10 mg/L Ciprofloxacin and 1.0% BSA):** Once opened the buffer is stable 3 months at 2-8°C. Prepare a new buffer working solution each day.
5. **Phospholipid:** Opened vial is stable for 3 months at 2-8°C. Shake gently before use.

Reagents and materials required but not provided

1. Deionized water, filtered through 0.22 mm or NCCLS type II water[11].
2. Acetic acid 20% or citric acid 2%.
3. Control Plasma Abnormal and Normal calibrated against an International Standard for Factor VIII
4. Calibration plasma calibrated against an International Standard
5. Photometer, 405 nm (and 490 nm for microplate procedure)
6. Heat incubator 37°C ± 0.2°C
7. Semi-micro cuvettes
8. Centrifuge, 2000xg
9. Plastic test tubes
10. Stopwatch
11. Vortex mixer
12. Calibrated pipettes

Specimen collection

Nine parts of freshly drawn venous blood are collected into one part trisodium citrate. Centrifugation: 2000 x g for 10-20 minutes at 20-25°C. Refer to NCCLS document H21-A4 for further instructions on specimen collection, handling and storage[12].

Quality Control

Normal and abnormal controls for plasma or concentrates are recommended for reliable quality control.[13] Assigned values of Controls should be traceable to the International Standard. Periodically within each run a control should be analyzed. The control material should be treated in the same way as a test sample. A range of allowable variation should be established for controls in each laboratory. If a value outside the established control range is obtained, a complete check of calibration, reagents and instrument performance should be made.

Results

Factor VIII results are reported in % activity (100% factor VIII activity is equivalent to 1.0 IU/mL).

Expected values

Range: 49 - 126 % (2 SD, n=121) in a normal healthy population evaluated with Coatest SP Factor VIII (test tube method).

Due to the many variables that may affect results, each laboratory should establish its own normal range, avoiding inadvertent losses of factor VIII activity.

Procedure

NOTE: All conditions included in this package insert refer to the manual method. Detailed settings for the ACL 8000/9000/10000 including instructions for preparation of the reagents are available on request from Chromogenix.

Two ranges of factor VIII are defined (20-150% and 1-20%).

Range 20-150%:
Prepare a solution of phospholipid+factor IXa and factor X reagent by mixing:

- 1 volume of phospholipid
- 5 volumes of factor IXa+factor X reagent

Keep on ice or at 2-8°C

Shake gently just before use.

The mixture is stable for 4 hours at 2-8°C or 12 hours on ice.

Calibration
A standard curve is required for each Coatest SP Factor VIII kit. Normal human plasma, calibrated against an International Standard, is used for preparation of standard dilutions in plastic tubes using pre-cooled buffer working solution according to the table below:

Predilution			Final dilution	
Standard %	Plasma µL	Buffer working-solution µL	Predilution µL	Buffer working-solution µL
150	-	undiluted	25	2000
120	200	50	25	2000
100	100	50	25	2000
75	100	100	25	2000
50	100	200	25	2000
21	100	600	25	2000

The assigned percentage values of the standard dilutions are those obtained from a normal plasma containing 1.0 IU factor VIII/mL. In case the factor VIII content of the normal plasma differs from this value, an appropriate correction factor should be used.

Preparation of plasma sample
Use plastic test tubes.
Test plasma or concentrate 25 µL
Buffer working solution (2-8°C) 3000 µL
Mix well. Keep at 2-8°C.
The assay must be performed within 30 minutes after dilution because of the lability of factor VIII.

Assay
NOTE: The assay should be performed in plastic material.

	Acid-stopped method	Initial rate method
Phospholipid+ FIXa+FX (2-8°C)	200 µL	200 µL
Test plasma or standard dilution (2-8°C)	100 µL	100 µL
Mix and incubate at 37°C 4-5 min		
$CaCl_2$ (37°C)	100 µL	100 µL
Mix and incubate at 37°C exactly 5 min		
S-2765+I-2581 (37°C)	200 µL	200 µL
Mix and incubate at 37°C exactly 5 min		
Acetic acid 20% or citric acid 2% (20-25°C)	100 µL	

Acid-stopped method: Read the absorbance of the sample against a reagent blank (buffer working solution instead of sample) within 4 hours.
Because of the large dilution of the plasma, no sample blanks have to be included.

Initial rate method: Transfer immediately to a 1 cm semi-micro cuvette (pre-heated to 37°C) and measure the absorbance change at 405 nm.

Range 1-20%:
Prepare a solution of phospholipid+factor IXa and factor X reagent by mixing:

- 1 volume of phospholipid
- 5 volumes of factor IXa+factor X reagent

Keep on ice or at 2-8°C.

Shake gently just before use.

The mixture is stable for 4 hours at 2-8°C or 12 hours on ice.

Calibration
A standard curve is required for each Coatest SP Factor VIII kit. Normal human plasma, calibrated against the International Standard, is used for preparation of standard dilutions in plastic tubes using pre-cooled buffer working solution according to the table below:

Standard %	Plasma µL	Buffer working-solution µL	Predilution µL	Buffer working-solution µL
20	50	200	25	2000
14.3	50	300	25	2000
9.1	50	500	25	2000
4.8	25	500	25	2000
1.2	25	2000	25	2000

The assigned percentage values of the standard dilutions are those obtained from a normal plasma containing 1.0 IU FVIII/mL. In case the FVIII content of the normal plasma differs from this value, an appropriate correction factor should be used.

Preparation of plasma sample
Use plastic test tubes.

Test plasma or concentrate	25 µL
Buffer working solution (2-8°C)	2000 µL

Mix well. Keep at 2-8°C.
The assay must be performed within 30 minutes after dilution because of the lability of factor VIII.

Assay
Because of the fairly small generation of FXa in samples with <5% factor VIII, the acid-stopped method is preferred for this range of factor VIII.

NOTE: The assay should be performed in plastic material.

Phospholipid+FIXa+FX (2-8°C)	200 µL
Test plasma or standard dilution (2-8°C)	100 µL
Mix and incubate at 37°C 4-5 min	
$CaCl_2$ (37°C)	100 µL
Mix and incubate at 37°C exactly 10 min	
S-2765+I-2581 (37°C)	200 µL
Mix and incubate at 37°C exactly 10 min	
Acetic acid 20% or citric acid 2% (20-25°C)	100 µL

Read the absorbance of the sample against a reagent blank (buffer working solution instead of sample) within 4 hours. Because of the large dilution of the plasma, no sample blanks have to be included.

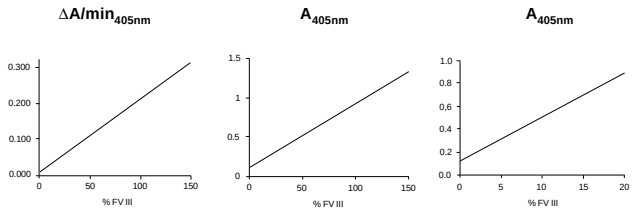
NOTE: The above described assay can also be conveniently performed in microplates by a four-fold reduction of all volumes, and keeping all other conditions such as incubation and hydrolysis times identical. In this case read the absorbance at 405 nm and 490 nm. Subtract the A_{490} from the A_{405} to correct for differences in microplate wells.

LIMITATIONS OF PROCEDURE
The activation reaction should be performed in plastic material since glass surfaces may interfere with the generation of factor Xa. Factor VIII is a labile coagulation factor and in order to obtain the accuracy which the method offers it is important to work in a carefully standardized manner throughout the assay procedure.

Calculation

Plot the change in absorbance per minute (ΔA/min) or absorbance (A) for the standards against their concentrations of factor VIII on linear graph paper. Read the % FVIII value for the corresponding absorbance for the unknown sample from the standard curve.

Standard Curves



Kinetic Method
Range 0-150 %

End Point Method
Range 0-150 %

End Point Method
Range 0-20 %

Specificity and Interfering Factors

FVIII results are not affected by Triglycerides at concentrations of 700 mg/dL, Bilirubin at concentrations of 20 mg/dL, Hemoglobin at concentrations of 100 mg/dL and unfractioned (UF) Heparin at concentrations of 1.0 IU/mL.

NOTE: Hemolyzed samples in the low range should not be analyzed.

Due to the high dilutions used, there is no underestimation of factor VIII activity in samples containing Lupus anticoagulant.

Precision
Within run and total precision was assessed over multiple runs.

System	%CV (Within run)	n	%CV (Total)	N
Test Tube method				
Mean FVIII				
83 %	3.4	2	5.3	80
14 %	4.3	2	5.6	80

Correlation:

System	Slope	Intercept	r	Reference method	n
Test Tube method	1.0851	-4.80	0.9873	Coatest Factor VIII (natural porcine phospholipid)	181

This study (n=181) was performed using samples from healthy individuals, as well as samples from patients with various levels of FVIII deficiency, von Willebrand's disease and other disorders.

Linearity

System	
Test Tube method:	0 -150% factor VIII

Detection Limit

System	
Test Tube method:	The assay allows detection of 1% factor VIII activity.

Sensitivity:

System	
Test Tube method	ΔA_{405} per 1% of FVIII activity: Low range 0.034 Normal range 0.009

Determinations/kit

Microplate method: 240 Test tube method: 60

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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