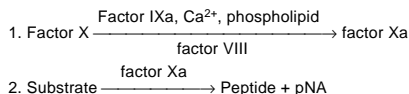


Intended Use

For the photometric determination of factor VIII activity in citrated plasma.

Measurement principle



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 Ca²⁺ 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-2222 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-2222 by thrombin formed is prevented by the addition of the synthetic thrombin inhibitor, I-2581, together with the substrate.

Composition

- S-2222 20 mg + I-2581** 1 vial
Chromogenic substrate (Bz-Ile-Glu(g-OR)-Gly-Arg-pNA), 20 mg and synthetic thrombin inhibitor, 335 µg with mannitol added as a bulking agent.
- Factor IXa + factor X 2.7 IU** 4 vials
Lyophilized bovine factors IXa and X with bovine albumin added as a stabilizing agent.
- CaCl₂ 6 mL** 1 vial
Calcium chloride solution, 0.025 mol/L.
- 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 and 0.2% BSA.
- Phospholipid 2 mL** 1 vial
Mixture of highly purified phospholipids.

PRECAUTION AND WARNINGS

Avoid contact with skin and eyes (S24/S25). 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:

- S-2222 + I-2581:** Reconstitute with 10.0 mL of sterile water or NCCLS type II water¹¹, to obtain a concentration of 2.7 mmol/L.
- Factor IXa + Factor X:** Reconstitute with 3.0 mL of sterile water or NCCLS type II water¹¹.
- CaCl₂:** Ready to use
- Buffer, stock solution:** Dilute 1:10 (1+9) with sterile water or NCCLS type II water¹¹. Prepare a new buffer working solution each day.
- 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.

- S-2222 + I-2581:** Stability after reconstitution: 6 months at 2-8°C.
- 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 1 month. Some activity (<10%) may be lost during freezing and thawing. The time needed to freeze the reagent should be as short as possible.
- CaCl₂ (0.025 mol/L):** Stable at 2-8°C until the expiry date printed on the label.
- Buffer, stock solution (Tris 0.05 mol/L, pH 7.3 and 0.2% Bovine albumin):** Once opened the buffer is stable 1 month at 2-8°C. Prepare a new buffer working solution each day.
- Phospholipid:** Opened vial is stable for 1 month at 2-8°C. Shake gently before use.

Reagents required but not provided

- Deionized water, filtered through 0.22 mm or NCCLS type II water¹¹.
- Acetic acid 20% or citric acid 2%.

- Normal human plasma. Prepare a normal plasma pool using the same procedure as for the plasma samples (see "SPECIMEN COLLECTION"). Blood samples are taken from at least 20 healthy donors. The plasma pool should be calibrated against the International Reference Plasma for FVIII Related Activities (available from National Institute for Biological Standards and Controls (NIBSC), London, UK).

Material required but not provided

- Photometer, 405 nm (and 490 nm for microplate procedure)
- Heat incubator 37°C ±0.2°C
- Semi-micro cuvettes
- Centrifuge, 2000xg
- Plastic test tubes
- Stopwatch
- Vortex mixer
- 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-A3 for further instructions on specimen collection, handling and storage¹².

Quality Control

Appropriate controls for plasma calibrated against an International Standard for Factor VIII should be used. Periodically within each run a control should be analysed. 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

Determination of factor VIII activity in 120 healthy individuals gave the following result: 88.9 ± 33.6 % (mean ± 2SD). Each laboratory should establish its own normal range, adhering to a standardized procedure and avoiding inadvertent losses of factor VIII activity.

Procedure

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 at 2-8°C. Shake gently just before use. The mixture is stable for 4 hours at 2-8°C.

Calibration

A standard curve is required for each Coatest VIII:C/4 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:

Standard %	Predilution		Final dilution	
	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 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

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-2222+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 at 2-8°C.

Shake gently just before use. The mixture is stable for 4 hours at 2-8°C.

Calibration

A standard curve is required for each Coatest VIII:C4 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 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.

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
CaCl₂ (37°C) 100 µL
Mix and incubate at 37°C exactly 10 min
S-2222+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.

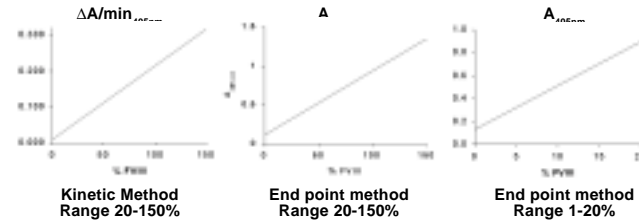
LIMITATIONS OF PROCEDURE

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 ($\Delta A/\text{min}$) 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



Performance Characteristics

Specificity and Interfering Factors

No drug interference reported.
Heparin concentrations ≤ 0.2 IU/mL do not interfere with the method, whereas 0.5 IU/mL gives 5% inhibition. Interference at high heparin levels is eliminated either by dilution or by addition of 10 μL Polybrene (1mg/mL) to each IU/mL of heparin. 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				
14.4 %	1.5	20	2.8	90
81.8 %	1.4	20	2.9	89

Correlation:

System	Slope	Intercept	r	Reference method	n
Test Tube method	1.002	0.365	0.985	Coatest Factor VIII (natural porcine phospholipid)	156

This study (n=156) 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 0.0393
Normal range 0.00859

Determinations/kit

Microplate method: 240 Test tube method: 60

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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
<i>In vitro</i> diagnostic medical device	Batch code	Use by	Temperature limitation	Consult instructions for use	Control	Biological risks	Manufacturer	Authorised representative
<i>In-vitro</i> Diagnostikum	Chargen-Bezeichnung	Verwendbar bis	Festgelegte Temperatur	Beilage beachten	Kontrollen	Biologisches Risiko	Hergestellt von	Bevollmächtigter
De uso diagnóstico <i>in vitro</i>	Identificación número de lote	Caducidad	Temperatura de Almacenamiento	Consultar la metódica	Control	Riesgo biológico	Fabricado por	Representante autorizado
Dispositif médical de diagnostic <i>in vitro</i>	Désignation du lot	Utilisable jusqu'à	Températures limites de conservation	Lire le mode d'emploi	Contrôle	Risque biologique	Fabricant	Mandataire
Per uso diagnostico <i>in vitro</i>	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 <i>in vitro</i>	Número de lote	Data limite de utilização	Limite 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
<i>In vitro</i> diagnostisk medicinsk produkt	Tillverkningskod	Användning	Temperatur gräns	Ta del av instruktionerna före användning	Kontrol	Biologiska risker	Tillverkare	Auktoriserad representant
Προϊόν για διαγνωστική χρήση <i>In vitro</i>	Αρ. Παρτίδας	Χρήση έως	Περιορισμοί θερμοκρασίας	Συμβουλευτείτε τις οδηγίες χρήσης	Υλικό ποιοτικού ελέγχου	Βιολογικοί κίνδυνοι	Κατασκευαστής	Εξουσιοδοτημένος αντιπρόσωπος