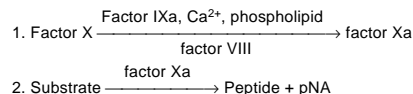


**Intended Use**

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 Ca<sup>2+</sup> 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 9.2 IU** 1 vial  
Lyophilized bovine factors IXa and X with bovine albumin added as a stabilizing agent.
- CaCl<sub>2</sub> 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 water<sup>11</sup>, to obtain a concentration of 2.7 mmol/L.
- Factor IXa + Factor X:** Reconstitute with 10.0 mL of sterile water or NCCLS type II water<sup>11</sup>.
- CaCl<sub>2</sub>:** Ready to use
- Buffer, stock solution:** Dilute 1:10 (1+9) with sterile water or NCCLS type II water<sup>11</sup>. 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.
- CaCl<sub>2</sub> (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 water<sup>11</sup>.
- 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 storage<sup>12</sup>.

**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 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:

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 <sub>2</sub> (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 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 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 CaCl<sub>2</sub> (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.

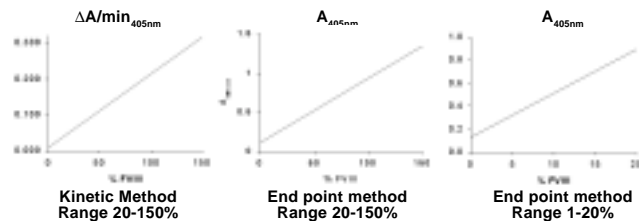
**LIMITATIONS OF PROCEDURE**

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 ( $\Delta A/min$ ) 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  $\leq 0.2$  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  $\mu$ L 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

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  $\Delta A_{405}$  per 1% of FVIII activity: Low range 0.0393  
Normal range 0.00859

**Determinations/kit**

Microplate method: 240 Test tube method: 60

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

1. Van Dieijen G et al. The role of phospholipid and factor VIII in the activation of bovine factor X. *J Biol Chem* 256, 3433-3442 (1981).
2. Mertens K et al: The role of factor VIII in the activation of blood coagulation factor X by activated factor IX. *Thromb Haemost* 54, 654-660 (1985).
3. Rosén S. Assay of factor VIII:C with a chromogenic substrate. *Scand J Haematol* 33, Suppl. 40 139-145 (1984).
4. Rosén S et al: Clinical application of a chromogenic substrate method for determination of factor VIII activity. *Thromb Haemost* 54, 818-823 (1985).
5. Lethagen S et al: Clinical application of the chromogenic assay of factor VIII in hemophilia A and different variants of von Willebrands disease. *Scand J Haematol* 37, 448-453 (1986).
6. Tripodi A, Mannucci P M. Factor VIII activity as measured by an amidolytic assay compared with a one-stage clotting assay. *Am J Clin Pathol* 86, 341-343 (1986).
7. Prowse C et al: Room temperature, microtray chromogenic assay of factor VIII:C. *Vox Sang* 50, 21-25 (1986).
8. Carlebjörk G et al: A simple and accurate microplate assay for the determination of factor VIII activity. *Thromb Res* 47, 5-14 (1987).
9. Koster T et al. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. *Lancet* 345, 152-155 (1995).
10. O'Donnell J et al. High prevalence of elevated factor VIII levels in patients referred for thrombophilia screening: role of increased synthesis and relationship to the acute phase reaction. *Thromb Haemost* 77, 825-828 (1997).
11. National Committee for Clinical Laboratory Standards. Specifications for reagent water used in the clinical laboratory, NCCLS Approved Standard: ASC-3.
12. National Committee for Clinical Laboratory Standards. Collection, transport and processing of blood specimens for coagulation testing and performance of coagulation assays, NCCLS Document H21-A3; vol. 18 No. 20. Dec. 1998

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

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