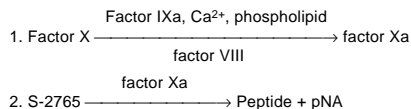


**Intended use**

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**



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-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**

- 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.
- 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, 10 mg/L Ciprofloxacin and 1.0% BSA.
- 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:

- S-2765 + I-2581:** Reconstitute with 12.0 mL of sterile water or NCCLS type II water<sup>11</sup>, to obtain a concentration of 1.8 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-2765 + I-2581:** Stability after reconstitution: 3 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 3 months. Do not refreeze.
- CaCl<sub>2</sub> (0.025 mol/L):** Opened vial is stable 3 months at 2-8°C.
- 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.

- Phospholipid:** Opened vial is stable for 3 months at 2-8°C. Shake gently before use.

**Reagents and materials 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%.
- Control Plasma Abnormal and Normal calibrated against an International Standard for Factor VIII
- Calibration plasma calibrated against an International Standard
- 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-A4 for further instructions on specimen collection, handling and storage<sup>12</sup>.

**Quality Control**

Normal and abnormal controls for plasma or concentrates are recommended for reliable quality control.<sup>13</sup> 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:

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 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+ F1Xa+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-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  $\mu$ L  
 Buffer working solution (2-8°C) 2000  $\mu$ L  
 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  $\mu$ L  
 Test plasma or standard dilution (2-8°C) 100  $\mu$ L  
 Mix and incubate at 37°C 4-5 min  
 CaCl<sub>2</sub> (37°C) 100  $\mu$ L  
 Mix and incubate at 37°C exactly 10 min  
 S-2765+I-2581 (37°C) 200  $\mu$ L  
 Mix and incubate at 37°C exactly 10 min  
 Acetic acid 20% or citric acid 2% (20-25°C) 100  $\mu$ L

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<sub>490</sub> from the A<sub>405</sub> to correct for differences in microplate wells.

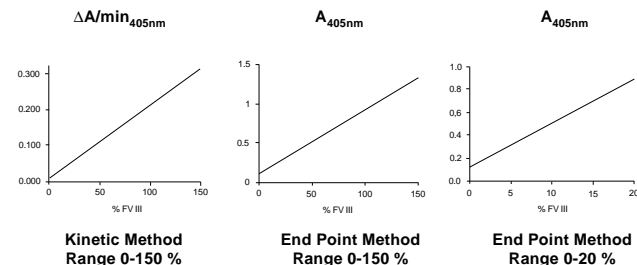
### LIMITATIONS OF PROCEDURE

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



### 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 unfractionated (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  $\Delta A_{405}$  per 1% of FVIII activity: Low range 0.034  
 Normal range 0.009






### 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 <i>In-vitro</i> 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 <i>In vitro</i> diagnostisk medicinsk produkt Προϊόν για διαγνωστική χρήση <i>In vitro</i>	Batch code Chargen-Bezeichnung Identificación número de lote Désignation du lot Número del lotto Número de lote Batch nr. Tillverkningskod Αρ. Παρτίδας	Use by Verwendbar bis Caducidad Utilizable jusqu'à Da utilizzzare prima del Data límite de utilização Anvendelse Användning Χρήση έως	Temperature limitation Festgelegte Temperatur Temperatura de Almacenamiento Températures limites de conservation Limiti di temperatura Límite de temperatura Temperatur begrænsninger Temperatur gräns Περιορισμοί θερμοκρασίας	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 Συμβουλευτείτε τις οδηγίες χρήσης	Control Kontrollen Control Contrôle Controllo Controlo Kontrol Kontroll Υλικό ποιοτικού ελέγχου	Biological risks Biologisches Risiko Riesgo biológico Risque biologique Rischio biologico Risco biológico Miljø oplysninger Biologiska risker Βιολογικοί κίνδυνοι	Manufacturer Hergestellt von Fabricado por Fabricant Prodotto da Fabricado por Producent Tillverkare Κατασκευαστής	Authorised representative Bevollmächtigter Representante autorizado Mandataire Rappresentanza autorizzata Representante autorizado Leverandør Auktoriserad representant Εξουσιοδοτημένος αντιπρόσωπος