COAMATIC® Factor VIII - 82 2585 63

ENGLISH - Insert revision 12/2002

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Intended use of the kit

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

Background and summary

The Coamatic Factor VIII kit provides reagents and chromogenic methods for determining factor VIII activity in human plasma and in factor VIII concentrates. Factor VIII is a high molecular weight plasma protein which serves as a cofactor to factor IXa in its activation of factor X to Xa.
Deficiency of factor VIII causes a severe bleeding disorder, hemophilia A. The severity of this bleeding disorder is inversely related to the factor VIII concentration. Hemophilia A patients are generally classified according to their factor VIII activity into three categories: <0.01 IU/mL = severe, 0.01-0.04 IU/mL =moderate and 0.05-0.25 IU/mL = mild hemophilia (4).

Measurement principle

In the presence of calcium ions and phospholipids, factor X is activated to factor Xa by factor IXa. This activation is greatly stimulated by factor VIII which acts as a cofactor in this reaction. By using optimal amounts of Ca^{2+}, phospholipid and factor IXa, and an excess of factor X, the rate of activation of factor X is linearly related to the amount of factor VIII. 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 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.

$$\text{Factor X} \xrightarrow[\text{Factor VIII}]{\text{Factor IXa, } Ca^{2+}\text{, Phospholipid}} \text{Factor Xa}$$

$$\text{S-2765} \xrightarrow{\text{Factor Xa}} \text{Peptide + pNA}$$

Composition

1. **S-2765 + I-2581** 1 vial
   Chromogenic substrate (N-a-Z-D-Arg-Gly-Arg-pNA) (7.7 mg) and synthetic thrombin inhibitor (0.2 mg) with mannitol (bulking agent).
2. **Factor reagent** 2 vials
   Bovine factor IXa (0.3 U) , factor X (2.7 IU) and thrombin (1 NIH-U) colyophilized with CaCl2 (40 mmol) and phospholipid (0.2 mmol).
3. **Buffer, stock solution** 1 vial
   24 mL concentrated Tris buffer, containing NaCl and BSA. Characteristics of diluted buffer (1:10): Tris 0.025 mol/L, pH 7.9, I = 0.08, 1% BSA.

The reagents are not interchangeable between lots.

PRECAUTIONS AND WARNINGS:
Harmful if swallowed (R 22). 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 6.0 mL of sterile water or NCCLS type II water [8]
2. **Factor reagent** reconstitute with 3.0 mL of sterile water or NCCLS type II water [8]
3. **Buffer, stock solution** dilute 1:10 (1+9) with sterile water or NCCLS type II water [8]

Reagent storage and stability

The sealed reagents are stable at 2-8°C until the expiry date printed on the label. Avoid contamination by microorganisms in the reagents.
1. **S-2765 + I-2581** Stability after reconstitution: 1 month at 2-8°C.
2. **Factor reagent** Stability after reconstitution: 12 hours at 2-8°C, 2 weeks at -30°C or 1 month at -70°C. Avoid storage at -20°C.
3. **Buffer, stock solution**. Stability after dilution: 1 month at 2-8°C.

Warning: Do not use reagents beyond the expiry date printed on the package label. If the substrate solution appears yellow, discard.

Specimen collection

Samples should be collected with the patient at rest and without stress. Sampling: Blood (9 vol) is mixed with 0.1 mol/L sodium citrate (1 vol) in plastic tubes or siliconized glass tubes. Centrifugation: 2000 x g for 10-20 minutes at 20-25°C. The plasma should be separated from the cells as soon as possible and the test should be performed within 30 minutes.

If the plasma is not tested immediately, store at -20°C for maximally one week or at -70°C for maximally one year. It should be noted that losses approaching 20% of the factor VIII activity may occur during freezing and thawing, especially if the plasma is frozen slowly. Specimens should not be stored in a self defrosting freezer and not be thawed and refrozen before assay.
Refer to NCCLS document H21-A2 for further instructions on specimen collection, handling and storage [9] .

Additional reagents

1. Deionized water, filtered through 0.22 mm or NCCLS type II water [8] .
2. Calibration plasma, calibrated against an International Standard
3. Control Plasma Abnormal and Normal, calibrated against an International Standard for Factor VIII
4. Saline (0.9% NaCl).
5. Acetic acid 20% or citric acid 2% (end-point methods).

Material required but not provided
— Spectrophotometer, 405 nm (and 490 nm for microplate procedure)
— Incubator 37°C ±+0.2
— Microplate or semi-micro cuvettes
— Centrifuge, 2000 x g
— Plastic test tubes
— Stopwatch
— Vortex mixer
— Calibrated pipettes
— Linear graph paper

Quality control

Appropriate controls for plasma or concentrates 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 IU/mL. 1.0 IU/mL of FVIII is equivalent to 100% FVIII.

Expected values

The Factor VIII levels measured in 61 healthy individuals, 28 males and 33 females, aged between 21 and 55, were in the range of 0.5-2 IU/mL. Median 1.13 IU/mL, SD 0.39 IU/mL. (Microplate method).

Procedures

All conditions included in this package insert are referred to Microplate method. Detailed instrument settings including instructions for preparation of the reagents for a variety of automated instruments are available on request from Chromogenix.

Calibration

A standard curve should be performed with every run. prepared by different dilution of Calibration Plasma which should be traceable to the International Standard.
A normal plasma can be also used for preparation of standard dilutions (it should be calibrated against an International Standard for plasma factor VIII) In case the normal plasma does not contain exactly 1 IU/mL (100%) factor VIII, the values of the standards must be recalculated accordingly, in order to obtain a correct factor VIII potency assignment.
This plasma is diluted according to a two-step procedure (predilution followed by final dilution) described in the below table. For factor VIII levels below 0.05 IU/mL (hemophilia A patients), the low range should be used.

	Prediluition		Final diluition	
Factor VIII IU/mL	Plasma µL	Buffer working solution µL	Plasma prediluition µL	Buffer working solution µL
Normal range				
1.42	undiluted	—	25	1400
1.00	undiluted	—	25	2000
0.50	100	100	25	2000
0.25	50	150	25	2000
0	—	—	—	2000
Low range				
0.050	100	1900	25	2000
0.024	50	2000	25	2000
0.012	25	2000	25	2000
0.006	25	4000	25	2000
0	—	—	—	2000

Assay condition for microplate and test tube techniques

The test should be performed within 30 minutes after sampling or thawing of the plasma samples.

Dilution of samples and controls:
Samples/controls 25 mL
Buffer working solution 2000 mL
Mix well

For extremely low levels of factor VIII, 0.005-0.05 IU/mL, a special range with increased reaction times is used in order to secure optimal resolution.

1. Microplate method

	Assay range 0-1.5 IU/mL	0-0.05 IU/mL
Diluted samples/controls/standards	50 µL	50 µL
incubate at 37°C	3-4 min	3-4 min
Factor reagent (pre-warm at 37°C)	50 µL	50 µL
incubate at 37°C	2 min	4 min
S-2765 + I-2581 (pre-warm at 37°C)	50 µL	50 µL

A. Kinetic method: read ΔA/min
B. End-point method: proceed as described below

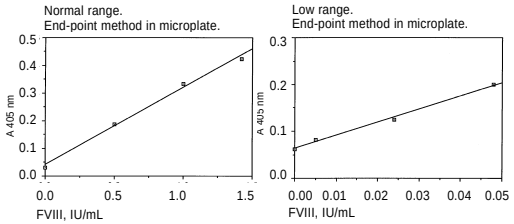
incubate at 37°C	2 min	10 min
Acetic add 20% or 2% citric acid	50 µL	50 µL

A. Kinetic method: read the absorbance change at 405 nm for 30-120 S.
B. End-point method: read the absorbance against buffer at 405 nm.

2. For manual method in plastic tubes, use 200 µL instead of 50 µL for all pipetting steps in the above scheme.

Calculation

Plot the change in absorbance per minute (ΔA/min) or absorbance (A) for the standard samples against their concentration of factor VIII on linear graph paper. Plot ΔA/min or A on the Y axis and IU/mL factor VIII on the X axis. Connect the standard points with the best fit straight line. Samples are evaluated from this standard curve. Examples of typical standard curves are shown below:



Absorbance values for the standard curve should be within the following limits:

Microplate method

	Standard	Kinetic, ΔA/min	End-point, A
Normal range	0 IU/mL	<0.06	<0.12
	1.0 IU/mL	0.16-0.32	0.33-0.63
Low range	0 IU/mL	<0.02	<0.18
	0.05 IU/mL	0.02-0.05	0.17-0.44
Test tube method			
Normal range	0 IU/mL	<0.14	<0.21
	1.0 IU/mL	0.38-0.74	0.58-1.11
Low range	0 IU/mL	<0.04	<0.32
	0.05 IU/mL	0.04-0.11	0.29-0.76

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Performance Characteristics

SPECIFICITY AND INTERFERING FACTORS

In order to minimize the influence from heparin in this assay, polybrene has been added to the reaction system. To prove the efficiency of this addition, plasma samples with heparin were used for standard preparation and standard curves were run in absence and presence of polybrene. In the presence of polybrene there is no inhibiting effect from 1.0 IU/mL heparin in the plasma. To check if polybrene by itself does not influence the system, standard curves were prepared from normal, heparin free plasma, in the absence and presence of polybrene. No influence from polybrene was detected in the case of heparin free plasma.

No other drug interference is reported.

Precision:
The following table shows the coefficient of variation (CV) for three different factor VIII plasma concentrations. NCCLS reference EP5-T2 User Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition; Tentative Guideline (1992). ISBN 1-56238-145-8.

Microplate method	CV% (Within series)	n	CV% (Between series)	n	N
Mean (FVIII IU/mL)					
1.0 IU/mL	2.4	42	2.1	6	7
0.25 IU/mL	2.6	42	5.9	6	7
0.03 IU/mL	3.0	42	3.9	6	7

n = number of replicates in each series, N = number of series

Correlation

The assay show a strong correlation with Coatest FVIII.

System	Slope	Intercept	r	Reference method	n
Manual Method	0.94	0.07	0.97	Coatest	25
Microplate	1.09	-0.11	0.96	Coatest	25
ACL	1.16	-0.13	0.97	Coatest	25
Cobas Mira	1.15	-0.10	0.98	Coatest	25
Hitachi	1.05	-0.09	0.98	Coatest	29
Electra MLA 900	1.03	-0.06	0.98	Coatest	25
Technicon RA-1000	0.95	-0.02	0.99	Coatest	25

Linearity

Two ranges of factor VIII are defined

System

Microplate and Manual method :
Normal range: 0.05-1.5 IU/mL Low range: 0.005-0.05 IU/mL

DETECTION LIMIT
System

Microplate and Manual method :
Normal range: 0.05 IU/mL Low range: 0.005 IU/mL

Sensitivity:
System

Microplate ΔAbs per 1IU/mL of FVIII activity

Normal range: 0.4Abs Low range 3.0Abs

Determinations/Kit

Microplate method: 120 Test Tube: 30

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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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