SIEMENS Healthineers

Factor VIII Chromogenic Assay

FACTOR VIII | CHROMOGENIC

Revision bar indicates update to previous version.

Intended Use

For photometric determination of Factor VIII (antihemophilic factor) activity in human plasma.

Summary and Explanation

Hemophilia is a sex-linked hemorrhagic disease caused by circulating Factor VIII (FVIII) or Factor IX (FIX) deficiency. Hemophilia A is the term used for a deficiency of FVIII, and is five to seven times more common than Hemophilia B, which is due to a deficiency in FIX[1–4].

Both diseases are transmitted as X-linked recessive traits and occur almost exclusively in males who receive the defective gene from the X-chromosome of a carrier mother. In Hemophilia A patients, the degree of FVIII deficiency dictates the severity of the bleeding disorder. Approximately 10 % to 40 % of normal FVIII activity is required for normal hemostasis; below this range, a tendency towards bleeding is apparent.

Patients are generally classified by their F. VIII activity into three categories: mild, 25 % to 5 % of normal; moderate, 5 % to 1 % of normal; and severe, less than 1 % of normal. In this chromogenic assay, the Factor VIII in the sample is activated by thrombin[5].

Activated Factor VIII (FVIIIa) then accelerates the conversion of Factor X (FX) into Factor Xa (FXa) in the presence of activated Factor IX (FIXa), phospholipids (PL) and calcium ions. The FXa activity is assessed by hydrolosis of a p-nitroanilide substrate specific to FXa. The initial rate of release of p-nitroaniline (pNA) measured at 405 nm is proportional to the FXa activity, thus to the FVIII activity in the sample. The chemistry of the FACTOR VIII | CHROMOGENIC is illustrated by the following equations:

$$\text{FVIII} + \text{Thrombin} \longrightarrow \text{FVIIIa}$$

$$\text{FX} \xrightarrow[\text{FXa, PL, } Ca^{2+}]{\text{FVIIIa}} \text{FXa}$$

$$CH_3OCO\text{-D-CHG-Gly-Arg-pNA} \xrightarrow{\text{FXa}} CH_3OCO\text{-D-CHG-Gly-Arg-OH} + \text{pNA (yellow)}$$

Reagents

Note: FACTOR VIII | CHROMOGENIC can be used manually or on automated coagulation analyzers. Siemens Healthineers provides Reference Guides (Application Sheets) for several coagulation analyzers. The Reference Guides (Application Sheets) contain analyzer/assay specific handling and performance information which may differ from that provided in these Instructions for Use. In this case, the information contained in the Reference Guides (Application Sheets) supersedes the information in these Instructions for Use. Please also consult the instruction manual of the instrument manufacturer!

Reagent	Description	Storage	Stability
Factor VIII Chromogenic Assay FACTOR VIII CHROMOGENIC			
REAGENT FX	Lyophilized reagent containing: • FX, bovine (reconstituted: ~2 nmol/vial) • Buffer: TRIS, pH 8 • Stabilizers • Preservative: Sodium azide (reconstituted: < 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 2 hours; 15–25 °C: reconstituted, 8 hours 2–8 °C: reconstituted, 3 days
REAGENT FIX	Lyophilized reagent containing: • FIXa, bovine (reconstituted: ~0.6 nmol/vial) • Thrombin, bovine (reconstituted: ~0.6 nmol/vial) • Calcium chloride (reconstituted: ~0.06 nmol/vial) • Phospholipids (reconstituted: ~0.12 µmol/vial) • Buffer: TRIS, pH 8 • Stabilizers • Preservative: Sodium azide (reconstituted: < 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 2 hours; 15–25 °C: reconstituted, 8 hours 2–8 °C: reconstituted, 3 days
SUBSTRATE	Lyophilized reagent containing: • CH_3OCO-D-CHG-Gly-Arg-pNA.AcOH (Factor Xa-Substrate, reconstituted: ~3.4 µmol/vial) • Nα-(2-Naphthylsulfonylglycyl)-D,L-amidinophenylalanine piperidide (α-NAPAP, a thrombin inhibitor)[6] • Stabilizers	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: reconstituted, 2 hours; 15–25 °C: reconstituted, 8 hours 2–8 °C: reconstituted, 3 days
SUBSTRATE BUFFER	Ready to use liquid containing: • Buffer: TRIS, EDTA, Sodium chloride • Preservative: Sodium azide (reconstituted: < 1 g/L)	2–8 °C May be used up to the expiry date indicated on the label if stored unopened.	37 °C: once opened, 2 hours; 15–25 °C: once opened, 8 hours 2–8 °C: once opened, 3 days

Signs of deterioration: No evidence of vacuum in vial upon opening; difficulty in reconstituting reagents; extreme turbidity or presence of particulate matter in SUBSTRATE BUFFER.

On-board stability
Information regarding on-board stability is specified in the Reference Guides (Application Sheets) for the different coagulation analyzers.

Warnings and Precautions

For *in-vitro* diagnostic use only.

For laboratory professional use.

Safety data sheets (MSDS/SDS) available on siemens-healthineers.com/sds.

CAUTION!
Federal (USA) law restricts this device to sale by or on the order of licensed healthcare professionals.

Warning! FACTOR VIII CHROMOGENIC REAGENT FIX
Hazardous ingredient: Calcium chloride (≥ 10 to ≤ 25 % [w/w]).
H319: Causes serious eye irritation.
P280: Wear protective gloves/protective clothing/eye protection/face protection. **P337 + P313**: If eye irritation persists: Get medical advice/attention.

Caution
FACTOR VIII CHROMOGENIC REAGENT FX, FACTOR VIII CHROMOGENIC REAGENT FIX
This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

Contains sodium azide as a preservative. Sodium azide can react with copper or lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides. Disposal into drain systems must be in compliance with prevailing regulatory requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with all government requirements.

Preparing Reagents

Important! For analyzer-specific information on reagent preparation please refer to the respective Reference Guides (Application Sheet) provided by Siemens Healthineers.

FACTOR VIII CHROMOGENIC REAGENT FX **and** FACTOR VIII CHROMOGENIC REAGENT FIX**:**
Dissolve the contents of a vial with 2.0 mL of distilled or deionized water.

FACTOR VIII CHROMOGENIC SUBSTRATE**:**
Dissolve the contents of a vial with 1.0 mL of distilled or deionized water.

FACTOR VIII CHROMOGENIC SUBSTRATE BUFFER**:**
For use, mix together 1.0 mL of the FACTOR VIII CHROMOGENIC SUBSTRATE with 10.0 mL of the FACTOR VIII CHROMOGENIC SUBSTRATE BUFFER in the brown FACTOR VIII CHROMOGENIC SUBSTRATE container.
Mix the reagents carefully once more before using.

Specimen Collection and Handling

Mix 9 parts of freshly collected patient blood with 1 part of 0.11 mol/L (3.2 %) or 0.13 mol/L (3.8 %) sodium citrate solution. Centrifuge the blood specimen at 1 500 × g for no less than 15 minutes at room temperature. Immediately move plasma into a plastic tube and keep refrigerated until ready to test.

Patient plasma should be tested within 1 hour of blood collection, and can be stored up to 30 days at –20 °C. Due to the instability of FVIII, the plasma may be thawed only once and tested within 1 hour.

Procedure

Materials Provided

REF	Contents		
B4238-40	Factor VIII Chromogenic Assay FACTOR VIII CHROMOGENIC		
	Factor X Reagent FACTOR VIII CHROMOGENIC REAGENT FX	2 × →	2 mL
	Factor IXa Reagent FACTOR VIII CHROMOGENIC REAGENT FIX	2 × →	2 mL
	Substrate Reagent FACTOR VIII CHROMOGENIC SUBSTRATE	2 × →	1 mL
	Stopping Buffer FACTOR VIII CHROMOGENIC SUBSTRATE BUFFER	2 ×	10 mL

Materials Required but not Provided

Item	Description
REF OTXW17	FACTOR VIII DEFICIENT, Coagulation Factor VIII Deficient Plasma (human)
REF ORKE45 REF OUPZ19	CONTROL N, Control Plasma N CONTROL P, Control Plasma P
REF B4234-25 REF B4265-37	OV BUFFER, Dade® Owren's Veronal Buffer or CA SYSTEM BUFFER, Dade® CA System Buffer
–	NaCl SOLUTION, isotonic Sodium Chloride Solution (0.9%)
–	For endpoint determination, 20 % acetic acid or 1 mol/L citric acid. (Mix 200 mL glacial acetic acid with 800 mL distilled or deionized water or dissolve 210 g citric acid monohydrate in distilled or deionized water and dilute to 1 liter.)
– REF ORKL19	Fresh plasma pool (FPP), or STANDARD PLASMA, Standard Human Plasma for calibration
Coagulation analyzers, such as:	• BCS® XP System • SYSMEX CA-1500 System • SYSMEX CS-2500 System • SYSMEX CS-5100 System

Additional Materials required for manual method

Item	Description
–	Distilled or deionized water
– –	Plastic tubes 1 cm semi-micro cuvettes
–	Pipettes for 0.1 mL, 0.5 mL and 3.0 mL
–	Stopwatch
Spectrophotometer	with the following capabilities: • Wavelength: 405 nm • Pathlength: 1 cm • Measuring temperatures: 25 °C, 30 °C or 37 °C

Test Procedure

Endpoint/Kinetic Assay Procedure

The assay can be performed at 25 °C, 30 °C or 37 °C. Set the wavelength of the photometer at 405 nm and zero against 0.9 % NaCl SOLUTION.

Sample/Calibration Plasma Dilution

The assay is performed on a 1:31 dilution of sample or calibration plasma. This dilution is prepared as follows: Mix 0.1 mL of sample or calibration plasma with 3.0 mL of 0.9 % NaCl SOLUTION in a plastic tube.

Preparation of the Calibration Curve:

The assay can be performed using a single calibration plasma dilution, but a calibration curve should be calculated for greater accuracy. For this, use a calibration plasma with known level of FVIII (e.g. 100 %) and dilute it with 0.9 % NaCl SOLUTION as follows:

Dilution of calibration plasma		1:21	1:31	1:62	1:124	zero standard
0.9 % NaCl SOLUTION	mL	2.0	3.0	1.0	1.0	1.0
Calibrating plasma	mL	0.1	0.1			–
Mix and transfer	mL		1.0	1.0		
				↑	↑	
FVIII[a]	%	148	100	50	25	0

[a] When a commercial assayed reference plasma is used, the percent of factor assumed present in the 1:31 dilution will be the assay value published. The percent of factor assumed present for the other dilutions must be adjusted accordingly. The FVIII concentration of the 1:21 dilution is calculated as follows: Concentration of FVIII in 1:31 dilution x 1.48 = Concentration of FVIII in 1:21 dilution, e.g. 100 % x 1.48 = 148 %.

Procedure Outline

All reagents as well as the plastic tubes should be preincubated at the selected temperature. The reproducibility is optimal if the measuring temperature does not deviate by more than 0.2 °C from the selected temperature during the reaction.

Step	Kinetic Assay	Endpoint Assay
Diluted sample/calibration plasma 1:31[b]	0.1 mL	0.1 mL
FACTOR VIII CHROMOGENIC REAGENT FX	0.1 mL	0.1 mL
FACTOR VIII CHROMOGENIC REAGENT FIX	0.1 mL	0.1 mL
Mix well and incubate	90 seconds at 37 °C[c]	
Add FACTOR VIII CHROMOGENIC SUBSTRATE and FACTOR VIII CHROMOGENIC SUBSTRATE BUFFER	0.5 mL	0.5 mL
Mix and measure the absorbance increase immediately (ΔA/min).	60 s 405 nm	60 s
Add 20 % acetic acid or 1 mol/L citric acid	-	0.1 mL
Mix and measure the absorbance against 0.9 % NaCl SOLUTION	-	405 nm

[b] See chapter titled "Notes on the Assay Procedure"

[c] 90 seconds at 37 °C, 120 seconds at 30 °C or 180 seconds at 25 °C.

For the endpoint assay a plasma bank must be prepared for the samples and each calibration plasma dilution.

20 % acetic acid	0.1 mL
Add FACTOR VIII CHROMOGENIC REAGENT FX. Mix.	0.1 mL
Add FACTOR VIII CHROMOGENIC REAGENT FIX. Mix.	0.1 mL

Add diluted Sample. Mix.	0.1 mL
Add FACTOR VIII CHROMOGENIC SUBSTRATE and FACTOR VIII CHROMOGENIC SUBSTRATE BUFFER. Mix.	0.5 mL
Mix and measure the absorbance against 0.9 % NaCl SOLUTION.	405 nm

Internal Quality Control

Normal range: CONTROL N

Pathological range: CONTROL P

Two controls should be measured with each calibration and at least every 3 to 4 hours during each testing day (one in the normal range and one in the pathological range). The controls should be processed just like the samples. Each laboratory should determine its own quality control range, either by means of the target values and ranges provided by the manufacturer of the controls or by means of its own ranges established in the laboratory. This range is usually based on ±2.0 to ±2.5 standard deviations (SD) from the mean control value. If the measured control value lies outside the confidence level previously established, then the coagulation analyzer, the reagents and the calibration should be examined. Do not release patient results until the cause of deviation has been identified and corrected.

Results

a) Using a single calibration factor (F)

Since the calibration curve is linear between 0 to 100 %, the FVIII activity in the sample can be calculated by using the known value of a calibration plasma (FPP, STANDARD PLASMA).

I. For kinetic assays

Percent FVIII Activity of Calibration Plasma = A

ΔA/min of the Calibration Plasma = C

ΔA/min of the Zero Standard = B

ΔA/min of the Sample = S

Calibration factor = F

$$\frac{A}{C - B} = F$$

The FVIII activity of the unknown sample in percent is calculated by the equation:

(S - B) x F = FVIII activity of the sample in %.

II. For endpoint assays

Percent FVIII Activity of Calibration Plasma = A

Calibration Plasma Absorbance = C

Calibration Plasma Blank Absorbance = C_b

Sample Absorbance = S

Sample Blank Absorbance = S_b

Zero Standard Absorbance = B

Zero Standard Blank Absorbance = B_b

Calibration factor = F

$$\frac{A}{(C - C_b) - (B - B_b)} = F$$

The FVIII activity of the unknown sample in percent is calculated by the equation:

$[(S - S_b) - (B - B_b)] \times F$ = Percent FVIII activity of the sample.

b) Using a Calibration Curve

I. For kinetic assays

Plot the FVIII activity in percent on the abscissa and the absorbance/minute measured at 405 nm, on the ordinate. Read the percent FVIII activity of the unknown sample from the calibration curve by finding the point where the ΔA/min intercepts the curve.

II. For endpoint assays

1. Subtract Calibration Plasma Blank Absorbance (C_b) from the Calibration Plasma Absorbance (C).
2. Subtract Sample Plasma Blank Absorbance (S_b) from Sample Absorbance (S).
3. Subtract the Zero Standard Blank Absorbance (B_b) from the Zero Standard Absorbance (B).

   Plot the FVIII activity in percent on the abscissa and (C-C_b) on the ordinate using linear graph paper.

   Read FVIII activity of the unknown sample from the calibration curve by finding the point where (S-S_b) intercepts the curve.

Limitations

Siemens Healthineers has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens Healthineers as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these instructions or use of the reagents on analyzers other than those included in Siemens Healthineers Reference Guides (Application Sheets) or these Instructions for Use.

Results of this test should always be interpreted in conjunction with the patient's medical history, clinical presentation and other findings.

Expected Values

FVIII levels of 60 to 168 % of Norm were determined in 124 ostensibly healthy individuals using the FACTOR VIII CHROMOGENIC on BCS®/BCS® XP.

For other coagulation instruments the expected values are 70 to 150 % of Norm[7].

It may be necessary to calculate your own reference range in the laboratory due to systematic differences in instrumentation.

Notes on the Assay Procedure

- The sensitivity of the assay in the low range of FVIII (0 to 10 % of Norm) may be improved if the FACTOR VIII DEFICIENT and FPP or STANDARD PLASMA are used as standards. Prepare 4 standards by mixing both plasmas accordingly in order to obtain 100 %, 50 %, 25 % and 0 % (FACTOR VIII DEFICIENT (human) alone). In this case, both standards and samples can be assayed at the 1:31 dilution.
- All reagents as well as the plastic tubes should be preincubated at the selected temperature. The reproducibility is optimal if the measuring temperature does not deviate by more than 0.2 °C from the selected temperature during the reaction.
- 1 mol/L citric acid can be used instead of 20 % acetic acid in the endpoint method.
- Disposable plastic materials are recommended for sampling and measuring.
- The reference curve must be re-determined for each change of device and for each new lot of FACTOR VIII CHROMOGENIC.

Performance Characteristics

In a comparative performance study, 98 patient samples were assayed by both the FACTOR VIII CHROMOGENIC and the coagulometric assay using deficient plasma and ACTIN. The correlation coefficient obtained was 0.96 and the regression equation was y = 1.0 × + 1.0 %.

Precision

The precision of the assay depends to some extent on the quality of the equipment and on the skill of the person performing the test. The following estimates of precision were made using the kinetic method at 37 °C.

Sample 1	n	Mean (%)	SD (%)	CV (%)
Between-run precision	11	118	5	4
Within-run precision	11	124	5	4

Heparin concentrates of up to 10 U/mL do not interfere with the FACTOR VIII CHROMOGENIC.

The linearity of the FACTOR VIII CHROMOGENIC is from 0 to 100 % FVIII[8].

Sample 2	n	Mean (%)	SD (%)	CV (%)
Between-run precision	11	25	2.5	10
Within-run precision	11	28	2.6	9

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens-healthineers.com

Current Version of Application Sheets

FACTOR VIII CHROMOGENIC can be used in combination with various automated coagulation analyzers. Siemens Healthineers provides Reference Guides/Application Sheets for the coagulation analyzers listed in section "Materials Required but not Provided", page 4 under the dedicated link below:

siemens-healthineers.com/rg

As Siemens Healthineers continuously monitors the product performance and safety, the users are required to ensure that they work with the correct revision of the instructions for the product lots in use. Please periodically review the availability of new electronic labeling revisions to ensure safe use of the product.

The IFU version number is visible on each product box label. Siemens Healthineers ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via siemens-healthineers.com/eIFU.

References

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5. van Dieijen G, Tans G, Rosing J, Hemker HC. The role of phospholipid and factor VIIIa in the activation of bovine factor X. J Biol Chem. 1981; 256: 3433-42.
6. Svendsen L, Brogli M, Lindeberg G, Stocker K. Differentiation of thrombin- and factor Xa-related amidolytic activity in plasma by means of a synthetic thrombin inhibitor. Thromb Res. 1984; 34: 457-62.
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Definition of Symbols

The following symbols may appear on the product labeling:

	Do not reuse		Use By
LOT	Batch Code	REF	Catalogue Number
	Caution		Manufacturer
EC REP	Authorized representative in the European Community		Contains sufficient for <n> tests
	Biological Risks	IVD	*In Vitro* Diagnostic Medical Device
	Temperature Limitation		Consult instruction for Use
	Non-sterile	CE	CE marking of conformity
CE 0197	CE marking of conformity with notified body ID number. Notified body ID number can vary.	CONTENTS	Contents
	Reconstitution volume	LEVEL	Level
	Keep away from sunlight and heat	WARNING	Warning
DANGER	Danger	RxOnly	Prescription device (US only)
UDI	Device Identification (UDI) barcode	REACH xx/xx/xx	REACH Authorization Number

Legal Information

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