

Factor VIII Chromogenic Assay

FACTOR VIII CHROMOGENIC

I Revision bar indicates update to previous version.

CE0197

Intended Use

FACTOR VIII CHROMOGENIC is an in vitro diagnostic reagent kit for the quantitative, WHO-standardized determination of coagulation factor VIII (FVIII) as aid to diagnosis and monitoring of congenital or acquired FVIII deficiencies in patients with bleeding disorders or at risk for FVIII deficiency in human sodium citrated plasma by means of automated, semi-automated and/or manual chromogenic methods.

In addition, the chromogenic FVIII assay can be used for monitoring FVIII substitution therapy.

Summary and Explanation

Hemophilia A is a congenital, FX-linked bleeding disorder caused by coagulation factor VIII (FVIII) deficiency. Acquired hemophilia A FVIII can develop due to presence of FVIII specific inhibitors. In the circulation FVIII is bound to its carrier protein von Willebrand factor (VWF). Both, FVIII and VWF are acute phase proteins, which increase in response to inflammation and stress^{1,2}.

FVIII is involved in the intrinsic coagulation pathway, FVIII deficiency causing prolongation of activated partial thromboplastin time (APTT). In contrast to the APTT-based one-stage clotting assay, the chromogenic FVIII assay represents a two-stage method: in the first step, FXa is generated; in the 2nd one, FXa is measured via a specific chromogenic substrate. The chromogenic assay offers a more standardized assay procedure with less influence of contact phase activation, amplification and propagation of clotting³⁻⁵.

The determination of FVIII activity in plasma is indicated in the following cases:

- clarifying the cause of a prolonged APTT,
- diagnosing congenital or acquired factor deficiency states,
- monitoring FVIII substitution therapy.

Reagents

Note: **FACTOR VIII CHROMOGENIC** can be used manually or on automated coagulation analyzers.

Sysmex 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!

Factor VIII Chromogenic Assay

Reagent	Description	Storage	Stability
Factor VIII Chromogenic Assay			
FACTOR VIII CHROMOGENIC			
REAGENT FX	Lyophilized reagent containing: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> 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: <ul style="list-style-type: none"> CH₃OCO-D-CHG-Gly-Arg-pNA.AcOH (Factor Xa-Substrate, reconstituted: ~3.4 µmol/vial) Na-(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: <ul style="list-style-type: none"> 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.

According to EU regulation 2017/746, any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State through your local distribution representative in which the user and/or patient is established.

Safety data sheets (MSDS/SDS) available upon request.



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 FIX**, **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.

Summary of Safety and Performance (SSP) is available in the European database on medical devices (see Eudamed public website: <https://ec.europa.eu/tools/eudamed>). In case Eudamed is not available, SSP can be delivered by the manufacturer on request.

Preparing Reagents

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

FACTOR VIII CHROMOGENIC **REAGENT FIX** 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 $1500 \times g$ 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] ORKE41	[CONTROL N], Control Plasma N
[REF] OUPZ17	[CONTROL P], Control Plasma P
[REF] B4234-25	[OV] [BUFFER], Dade® Owren's Veronal Buffer or
[REF] B4265-37	[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.)
–	Fresh plasma pool (FPP), or
[REF] ORKL17	[STANDARD PLASMA], Standard Human Plasma for calibration
Coagulation analyzers ^a , such as:	<ul style="list-style-type: none"> AUTOMATED BLOOD COAGULATION ANALYZER CS-2500 (CS-2500 System) AUTOMATED BLOOD COAGULATION ANALYZER CS-5100 (CS-5100 System)

^a Availability of analyzers may vary by country.

Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified.

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: <ul style="list-style-type: none"> Wavelength: 405 nm Pathlength: 1 cm Measuring temperatures: 25 °C, 30 °C or 37 °C

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

$\Delta A/\text{min}$ of the Calibration Plasma = C

$\Delta A/\text{min}$ of the Zero Standard = B

$\Delta A/\text{min}$ 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:

$(S - B) \times F = \text{FVIII activity of the sample in \%}$.

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:

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

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 $\Delta A/\text{min}$ 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

The manufacturer has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. Please note that the applications on other analyzers can be validated by the instrument manufacturer in accordance with the requirements of the REGULATION (EU) 2017/746 under their responsibility as long as the intended purpose and performance are not modified. User defined modifications are not supported by the manufacturer 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 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 Norm⁷.

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 Assay and the coagulometric assay using deficient plasma and **ACTIN** (Dade[®] Actin[®] Activated Cephaloplastin Reagent). The correlation coefficient obtained was 0.96 and the regression equation was $y = 1.0 \times + 1.0 \%$.

Measuring Range

The measuring range depends on the individual application of the assay due to instrument related conditions. Application specific performance data is listed in the respective Reference Guides of the instruments.

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 Assay. The linearity of the Factor VIII Chromogenic Assay is from 0 to 100 % FVIII⁸.

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

Other system specific results are given in the respective Reference Guides (Application Sheets).

The reproducibility was assessed by the manufacturer for **FACTOR VIII DEFICIENT** based on proficiency testing information in 2019/2020. The overall reproducibility median CV% was found to be 10 % including lot, instrument, laboratory and operator variability factors.

Technical Assistance

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

Current Version of Application Sheets

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

sysmex-ifu.com/ag

As the manufacturer 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. Sysmex ensures that all products lots bearing the same IFU version number are compatible with the electronic labeling provided via sysmex-ifu.com.


























References

1. Bolton-Maggs PH, Pasi KJ. Haemophilias A and B. Lancet. 2003;361:1801-9.
2. Franchini M, Mannucci PM. Acquired haemophilia A: A 2013 update. Thromb Haemost. 2013;110:1114-20.
3. Adcock DM, Strandberg K, Shima M, et al. Advantages, disadvantages and optimization of one-stage and chromogenic factor activity assays in haemophilia A and B. Int J Lab Hematol. 2018;40:621-629.
4. Castellone DD, Adcock DM. Factor VIII Activity and Inhibitor Assays in the Diagnosis and Treatment of Hemophilia A. Semin Thromb Hemost. 2017;43:320-30.
5. Bowyer AE, Duncan EM, Antovic JP. Role of chromogenic assays in haemophilia A and B diagnosis. Haemophilia 2018; 24:578-583.
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.

7. Fickenscher K. Analysis of individual coagulation factors. In: Thomas L, editor. Clinical Laboratory Diagnostics. Frankfurt: TH-Books Verlagsgesellschaft; 1998: 607-9.
8. Tran TH, Zuehlke U, Hauert J, et al. Influence of heparin on factor VIII (FVIII) assay. Thromb Haemost. 1987; 58: 341 (Abstract).


Definition of Symbols

The following symbols may appear on the product labeling:

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

Legal Information

Sysmex is a trademark of SYSMEX CORPORATION.
 Actin, BCS and Dade are trademarks of Siemens Healthineers.
 All other trademarks are the property of their respective owners.
 © Siemens Healthineers, 2009–2024. All rights reserved.

 **Siemens Healthcare Diagnostics Products GmbH**
 Emil-von-Behring-Str. 76
 35041 Marburg
 Germany
[siemens-healthineers.com](https://www.siemens-healthineers.com)