BIOPHEN™ FVIII variant

REF 227102-RUO

R1 R2 R3 2 vials x 2.5 mL

R4 4 vials x 20 mL

FOR RESEARCH USE ONLY.
DO NOT USE IN DIAGNOSTIC PROCEDURES.

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INTENDED USE:

Chromogenic method for the *in vitro* quantitative determination of Factor VIII (FVIII) activity, in human citrated plasma or FVIII concentrates, using a manual or automated method.
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:[1-5]
Factor VIII is a heterodimeric glycoprotein consisting of a metal ion-linked light chain and a heavy chain and circulates in plasma at a concentration of approximately 200 ng/mL. FVIII interacts non-covalently with von Willebrand Factor which dramatically prolongs its half-life in blood circulation. Activated FVIII accelerates more than 100 000-fold the Factor X activation in the presence of Factor IXa, phospholipids and calcium ions.

PRINCIPLE:

The BIOPHEN™ Factor VIII variant method involves the chromogenic assay of FVIII cofactor. In the presence of phospholipids (PLPs) and calcium, FVIII, activated by thrombin, forms an enzyme complex with Factor IXa, which activates Factor X. The resulting Factor Xa hydrolyzes the chromogenic substrate, leading to the release of paranitroaniline (pNa). The amount of pNA released (measured by absorbance at 405 nm) is directly proportional to the concentration of FVIII in the specimen (Factor IXa, Thrombin and Factor X are in constant excess amount).
The BIOPHEN™ FVIII variant kit is a variant method of BIOPHEN™ FVIII (221402-RUO / 221406-RUO) where FX is from bovine origin and which is insensitive to Emicizumab.

REAGENTS:

R1 **Bovine Factor X** at approximately 7.5 µg/mL, lyophilized. Contains a fibrin polymerization inhibitor, BSA and stabilizers.
R2 **Activation Reagent,** human Factor IIa at approximately 1 NIH/mL, human Factor IXa at approximately 2 µg/mL and synthetic Phospholipids (1:40 dilution), lyophilized. Contains BSA, Calcium Chloride Dihydrate and stabilizers.
R3 **SXa-11,** chromogenic substrate specific to Factor Xa (CS-11(32)) at approximately 6 mg/mL, lyophilized. Contains stabilizers and EDTA disodium salt.
H373: May cause damage to organs through prolonged or repeated exposure.
R4 **Tris-BSA Buffer,** liquid. Contains BSA, human acid glycoprotein (AGP), stabilizers and preservatives.

WARNINGS AND PRECAUTIONS:

- Some reagents provided in these kits contain materials of human and animal origin. Whenever human plasma is required for the preparation of these reagents, approved methods are used to test the plasma for the antibodies to HIV 1, HIV 2 and HCV, and for hepatitis B surface antigen, and results are found to be negative. However, no test method can offer complete assurance that infectious agents are absent. Therefore, users of reagents of these types must exercise extreme care in full compliance with safety precautions in the manipulation of these biological materials as if they were infectious.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits.

REAGENT PREPARATION:

Gently remove the freeze-drying stopper, to avoid any product loss when opening the vial.

R1 R2 R3 Reconstitute the contents of each vial with exactly **2.5 mL of distilled water.**
Shake vigorously until **complete dissolution** (ensure that there is no deposit at the bottom of the R3 vial, if necessary, let each R3 vial stabilize for at least 15 minutes at 37°C and homogenize before use) while avoiding formation of foam and load it directly on the analyzer following Application Guide instruction.
For manual method, allow to stabilize 30 min at room temperature (18-25°C), homogenize before use.

R4 Reagent is ready to use; homogenize while avoiding formation of foam and load it directly on the analyzer following Application Guide instruction.
For manual method, allow to stabilize for 30 minutes at room temperature (18-25°C), homogenize before use.

STORAGE AND STABILITY:

Unopened reagents should be stored at 2-8°C in their original packaging. Under these conditions, they can be used until the expiry date printed on the kit.

R1 R2 R3 R4 Reagent stability after reconstitution / opening, free from any contamination or evaporation, and stored closed, is of:

- **72 hours** at 2-8°C.
- **Stability on board of the analyzer: see the specific Application Guide.**

Combination of storage are not recommended.

If the substrate becomes yellow, this indicates a contamination. Discard the vial and use a new one.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:

- Specific calibrators and controls with known titration, such as:

Product Name	Reference
BIOPHEN™ Plasma Calibrator	222101-RUO
BIOPHEN™ Normal Control Plasma	223201-RUO
BIOPHEN™ Abnormal Control Plasma	223301-RUO

- For low-range calibration, dilute the calibrator in Factor VIII Deficient Plasma (DP040A-RUO/DP040K-RUO, HYPHEN BioMed).
- Automatic analyzer for chromogenic assays such as: CS-series.
- Laboratory material.

SPECIMEN COLLECTION AND PREPARATION:

The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.
Samples should be collected, prepared and stored in accordance with applicable local guidelines (for the United States, see the CLSI H21-A5[6] guideline for further information concerning specimen collection, handling and storage).
For plasma storage, please refer to references[6,7,8].

PROCEDURE:

Automated method:
For the low range, the 6 calibration points should be prediluted in Factor VIII Deficient Plasma to obtain the calibration range from about 0.9% to 28% before loading onto the analyzer.

HYPHEN BioMed provides Application Guides for defined coagulation analyzer families. The Application Guides contain analyzer/assay specific handling and performance information and supersede the information in these Instructions for Use.

For FVIII concentrates, pre-dilute the test specimen (**high range**) in R4.

Assay method:
1. Reconstitute the calibrators and controls as indicated in the specific instructions. Calibrators should be diluted in the R4 buffer as described in the table below in order to prepare the calibration curve ("C" defines the concentration of FVIII).

High range (0 to 200%):
When the calibration curve is established using a commercial calibrator plasma (e.g.: BIOPHEN™ Plasma Calibrator), the **1:40** dilution corresponds to the indicated concentration (**C**) of FVIII and the **1:20** dilution to twice this concentration. For a calibrator with a titer of **C**, the 200% level (under assay conditions) is obtained by diluting this calibrator by the following factor: **20x(C)/100**.
The calibration curve can also be established using a pool of citrated normal plasmas (at least 30 normal individuals, men and women, aged between 18 and 55 years, with no known treatments or diseases), which, by definition, has a FVIII titer of **100%**. The assay includes a **1:40** plasma dilution, which by definition, represents the **100%** FVIII level. The calibration curve ranges from **0 to 200%** FVIII. The **1:20** dilution in R4 buffer represents **200%** FVIII.

Prepare **1 mL** of the **1:20** normal plasma pool dilution, or a **(20xC/100)** dilution of the FVIII titrated calibrator plasma (i.e. **C1**). This solution has a FVIII titer of 200%. Prepare the following calibration curve by serial dilution in R4 buffer, as described in the following table:

Calibrator	C1	C2	C3	C4
FVIII (%)	**200**	**100**	**50**	**0**
Volume of 200% FVIII Calibrator	500 µL	250 µL	125 µL	0 µL
Volume of R4 buffer	0 µL	250 µL	375 µL	500 µL

The calibration curve can also be established from a FVIII titrated reference material (international standard or internal standard).
Pre-dilute this material in R4 buffer to obtain a **1 IU/mL** solution, then dilute **1:20** in R4 to obtain a solution with a **200%** (**2 IU/mL**) FVIII titer. Use this solution to establish a calibration curve in R4 buffer as previously explained.

Low range (0 to 25%):
Calibration can be performed using a pool of citrated normal plasmas, or a commercial calibrator plasma with a known concentration of FVIII, i.e. **C**. Dilute this plasma in Factor VIII Deficient Plasma (DP040A-RUO / DP040K-RUO) to achieve a **25%** concentration (the dilution factor in deficient plasma is of **4** for the normal pool and of **4xC:100** for a calibrator with a concentration **C**).
The assay method includes a **1:10** plasma dilution. The calibration curve ranges from **0 to 25%** FVIII. The **1:10** dilution in R4 buffer represents **25%** FVIII.

Using this solution, establish the following calibration curve in **R4** buffer:

FVIII (%)	0	6.25	12.5	25
Volume of 25% FVIII Calibrator	0 µL	125 µL	250 µL	500 µL
Volume of **R4** buffer (1)	500 µL	375 µL	250 µL	0 µL

(1) When assaying very low levels, dilution in Factor VIII Deficient Plasma may be used instead of **R4** buffer to prepare the calibration points (eg. See section automated method procedure and Application Guide).

Prepare the calibration curve immediately before use to avoid any FVIII degradation.

2. Dilute the specimens in **R4** buffer, as described in the table below:

Specimens	Reference	Range	Dilution
Control	223201-RUO/ 223301-RUO	**High**	**1:40**
		Low (after 1:10 pre-dilution in FVIII-deficient plasma)	**1:10**
Specimens	N.A.	**High**	**1:40**
		Low	**1:10**

For FVIII concentrates, pre-dilute the test specimen (**high range**) in **R4**, aiming for a FVIII concentration of approximately 1 IU/mL. We recommend performing a pre-dilution, in order to adjust the theoretical FVIII concentration to **between 0.2 and 2 IU/mL**, then dilute **1/40** in **R4** to perform the test. The expected FVIII concentration is thus of between 20 and 200%.
(the measured concentration should then be multiplied by the "pre-dilution" factor).

Establish the calibration curve and test it with the quality controls. If stored at room temperature (18-25°C), test the diluted specimens quickly. The exact calibrator and control concentrations for each batch are indicated on the flyer provided with the kit.

3. Dispense the following to the wells of a microplate, or to a plastic tube incubated at **37°C**:

Reagents	Microplate	Test Tube
Calibrators, diluted tested plasmas, or Controls	50 µL	100 µL
R1 **Bovine Factor X**, preincubated **at 37°C**	50 µL	100 µL
R2 **Activation Reagent,** preincubated **at 37°C**	50 µL	100 µL
Mix and incubate at **37°C for 2 minutes,** then add the following:		
R3 **SXa-11**, preincubated **at 37°C**	50 µL	100 µL
Mix and incubate at **37°C** for **3 minutes exactly**		
Stop the reaction by adding:		
Citric Acid (2%)*	50 µL	200 µL
Mix and measure the optical density at **405nm** against the corresponding blank.		

*Or acetic acid (20%). The yellow color is stable for 2 hours.
The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Citric acid (2%), R3, R2, R1, dilute specimen**.**
Measure the optical density at 405 nm. Subtract the measured blank value from the absorbance measured for the corresponding test.

QUALITY CONTROL:

The use of quality controls serves to validate method compliance, along with between-test assay homogeneity for a given batch of reagents.
Include the quality controls with each series, as per good laboratory practice, in order to validate the test. A new calibration curve should be established, preferably for each test series, and at least for each new reagent batch, or after analyzer maintenance, or when the measured quality control values fall outside the acceptance range for the method.
Each laboratory must define its acceptance ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve log-log (high range) and lin-lin (low range), with the OD 405nm along the Y-axis and the FVIII concentration, expressed as %, along the X-axis.
- The concentration of FVIII (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.
- For FVIII concentrates, the measured concentration should then be multiplied by the "pre-dilution" factor.
- Lot to lot variability measured on 3 lots is: %CV ≤ 10%.

The results obtained should be for research use only and must not be used for patient diagnosis or treatment.

LIMITATIONS:

- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.
- Any reagent presenting no limpid appearance or showing signs of contamination must be rejected.
- Any suspicious samples or those showing signs of activation must be rejected.
- User defined modifications are not supported by HYPHEN BioMed 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 HYPHEN BioMed Application Guides or these Instructions for Use.

PERFORMANCES:

Mathematical analyses are performed using a validated statistical software built in accordance with CLSI guidelines.
Performances studies were conducted as described in CLSI guidelines.
The following performance data represent typical results and are not to be regarded as specifications for BIOPHEN™ FVIII variant.

Analytical performances
Measuring Range (on CS-series)

Limit of Blank (LOB)	High range		4.0%
	Low range		0.0%
Limit of Detection (LOD)	High range		4.4%
	Low range		0.0%
Limit of Quantification (LOQ)	Direct	High range	25% - 185%
		Low range	0.9% - 27%
	With re-dilution	High range	4.5% - 460%

Precision (on CS-series)

Samples	Repeatability			Within-Laboratory	
	n	Mean (%)	CV (%)	Mean (%)	CV (%)
Normal Control (High range)	20	112.5	0.5	112.5	2.2
Abnormal Control (High range)	20	39.3	1.0	39.3	3.3
Normal Control (Low range) (pre-diluted 1:10)	20	12.0	0.7	12.0	2.9
Abnormal Control (Low range) (pre-diluted 1:10)	20	4.4	1.4	4.4	5.3

Interfering substances
No interferences, on CS-series, up to :

Bilirubin C	45 mg/dL	**Dabigatran**	Sensitive
Bilirubin F	45 mg/dL	**Apixaban**	Sensitive
Intralipids	1000 mg/dL	**Rivaroxaban**	Sensitive
Hemoglobin	1000 mg/dL	**Edoxaban**	Sensitive
UFH	Sensitive	**Emicizumab**	94 µg/mL
LMWH	Sensitive		

This FVIII assay is insensitive to Emicizumab[9].

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The following symbols may appear on the product labeling:

REF	Catalogue number	**LOT**	Batch code	**RUO**	Product for *in-vitro* research use, only
Rx	Numerical < x> identification of reagent		See instructions for use	**WHO STD**	WHO standard code
	Temperature limitation		Manufacturer	YYYY-MM-DD	Use by
	Biological risks	→	Reconstitution volume	**CONTENTS**	Contents
Cx	Numerical < x> identification of control	**i-MA**	See instructions in Method Application guide	**CONTAINS**	Contains
EXP	Expiration date	Σ	Contains sufficient for <n> tests	**UNIT**	Measurement unit
TARGET VALUE	Target Value		Keep away from sunlight and heat	**CALx**	Numerical < x> identification of calibrator
ACCEPTANCE RANGE	Acceptance range				

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