HYPHEN BioMed
155 rue d'Eragny, 95000 Neuville-sur-Oise, France
Tél : +33 (0)1 34 40 65 10
Fax : +33 (0)1 34 48 72 36
www.hyphen-biomed.com
info@hyphen-biomed.com

CE 0197 UK CA

IVD

BIOPHEN™ FVIII

REF 221402 R1 R2 R3 2 vials x 2.5 mL,
R4 4 vials x 25 mL
REF 221406 R1 R2 R3 2 vials x 6 mL,
R4 4 vials x 25 mL

English, revision: 12-2025

INTENDED USE:

Chromogenic method for the *in vitro* quantitative determination of Factor VIII (FVIII) activity, in human citrated plasma or therapeutic concentrates, using an automated method. This method is an aid to diagnosis of FVIII deficiency states and monitoring of replacement therapy on patients who are suspected of congenital or acquired deficiency and potency estimation of FVIII concentrates.
This device of *in vitro* diagnostic use is intended for professional use in the laboratory.

SUMMARY AND EXPLANATION:

Technical:[1-6]
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.
Clinical:[5-11]
Hemophilia A is an X-linked recessive disorder leading to a deficiency of functional FVIII. Bleeding tendency is directly linked to the level of FVIII activity and patients are classified as mild (5% to 40% of FVIII), moderate (1% to 5% of FVIII), or severe (<1% of FVIII). Treatment for hemophilia consists of replacing the missing clotting factor (FVIII) on a regular basis (prophylaxis) or episodically (on-demand treatment). FVIII levels are reduced in von Willebrand's disease (vWD) or in case of Disseminated Intravascular Coagulation (DIC) or acquired FVIII inhibitor. Elevated concentrations of FVIII are observed in inflammatory or hepatic diseases and may be suggestive of an increased risk of venous thrombosis.

PRINCIPLE:

The BIOPHEN™ Factor VIII 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).

REAGENTS:

R1 **Human 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 Phospholipids (about 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. **Warning!** 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.
- Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
- Use only the reagents from the same batch of kits.
- 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 or on request to HYPHEN BioMed).
- Please consult Safety Data Sheet (SDS), available on www.hyphen-biomed.com.P260 : Do not breathe dust/fume/gas/mist/vapours/spray.
- P314 : Get medical advice/attention if you feel unwell.

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 :
REF 221402 ➔ R1 R2 R3 2.5 mL of distilled water
REF 221406 ➔ R1 R2 R3 6 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.

R4 Reagent is ready to use; homogenize while avoiding formation of foam and load it directly on the analyzer following Application Guide instructions.

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.**

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:

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

- For low-range calibration, dilute the calibrator in Factor VIII Deficient Plasma (DP040K, HYPHEN BioMed).
- Optional when required, Special Tris-BSA buffer (AR026K). Use the same buffer for all dilutions performed.
- Automatic analyzer for chromogenic assays such as: CS-series, STA-R® family, ACL-TOP® family, CN-series.
- Laboratory material.

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 is not modified.

TRACEABILITY:

Certificates of traceability and Instructions for Use of above calibrators and controls are available on the HYPHEN BioMed website. For more information refer to Instructions for Use of above calibrators and controls.

SPECIMEN COLLECTION AND PREPARATION:

Collection, preparation and storage of Platelet Poor Plasma (PPP) should be made according to laboratory or other validated methods[12-15].
The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 mol/L, 3.2%) by clean venipuncture.
CLSI H21-Ed6[12] and studies[11,15]:

- Plasma should remain at room temperature for no longer than 4 hours.
- If assays will not be completed within 4 hours, plasma should be frozen at −20 °C or below.
- Plasma samples should be thawed at 37°C, only once.

PROCEDURE:

HYPHEN BioMed provides Application Guides for defined coagulation analyzer families. The Application Guides contain analyzer/assay specific handling and

performance information and complement the information in these Instructions for Use.

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

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:

- The concentration of FVIII (%) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used (reported in IU/mL, or % considering 100%=100 IU/dL= 1IU/mL).
- 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 ≤ 4%.
- The results should be interpreted according to the patient's clinical and biological condition, and other findings.

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.
- FVIII assay could be sensitive to anticoagulant drugs and is sensitive to bispecific antibodies mimicking FVIII[16].
- Depending on the variant and concentrate, discrepancy between chromogenic and clotting assays is reported[5,6,9,12, 17].
- Results might be increased due to patient's conditions and treatment (e.g. stress, pregnancy, patient under desmopressin acetate, etc.)[12].

EXPECTED VALUES:

The reference interval established on healthy adult subjects, in internal study, on CS-series (n=134), STA-R® family (n=131), ACL-TOP® family (n=126), CN-series (n=131) was measured between 66 and 146 %, between 60 and 142 %, between 62 and 148 % and between 65 and 138 % respectively (Central 90%, 95th percentile).
However, each laboratory has to determine its own normal ranges[12-15].
A normal range study was performed on each analyzer and is documented in the respective Application Guides of the analyzers.

PERFORMANCES:

Mathematical analysis 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.
All performances are documented in the respective Application Guides of the analyzers.

Analytical performances
Measuring Range
The measuring range is defined by the analyzer system used and is documented in the respective Application Guides of the analyzers.
Accuracy
Accuracy studies were assessed using laboratory controls and frozen plasmas.
Trueness: bias is less than 12% for all samples.
Precision: Coefficient of variation (CV) for all samples is less than 10% for repeatability, less than 10% for reproducibility and less than 10% for within laboratory. Precision is documented in the respective Application Guides of the instruments.
Interfering substances
Interferences are defined by the analyzer system used and are documented in the respective Application Guides of the analyzers.

Clinical performances
Clinical study was performed on 206 plasmas samples from healthy subjects (120) and from patients with Haemophilia A (86).

Agreement

	ACL TOP® family			
Analyte	**n**	**Linear regression**	**r**	**Reference / comparison method**
Factor VIII	206	y = 0.97x + 1.24	0.975	HemosIL® SynthASil (HemosIL® Factor VIII deficient plasma)

Sensitivity/Specificity

	ACL TOP® family				
Analyte	**n**	**Sensitivity**	**Specificity**	**Area under the curve (ROC)**	
Factor VIII	206	0.933	1.000	1.000	
Analyte	**n**	**PPV**	**NPV**	**LR+**	**LR-**
Factor VIII	206	100%	93%	+∞	0.06

PPV: Predictive value of a positive result LR+ : Likelihood Ratio +
NPV: Predictive value of a negative result LR- : Likelihood Ratio –

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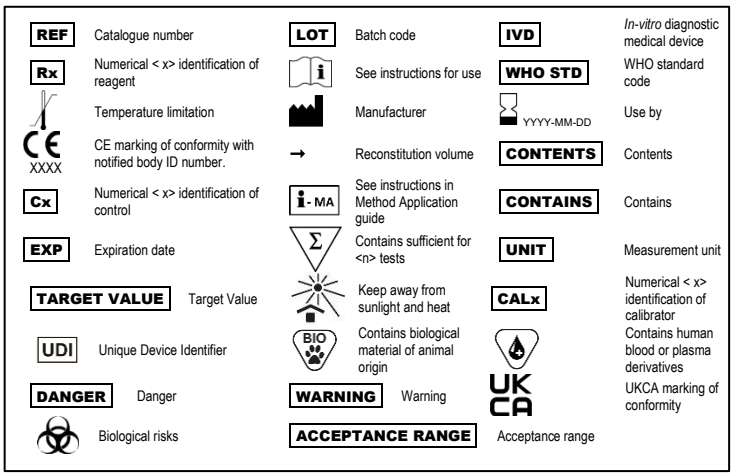
e-IFU and SDS (other languages) are available on www.hyphen-biomed.com.
For customer support and Application Guides, please contact your local provider or distributor (see www.hyphen-biomed.com).

Changes compared to the previous version.

The following symbols may appear on the product labeling:



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