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

BIOPHEN™ DiXal

REF 221030-RUO

R1 R2 3 vials x 2.5 mL

R3 4 vials x 20 mL

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

English, revision: 12-2023

INTENDED USE:

Anti-Xa chromogenic method for the *in vitro* quantitative determination of direct Factor Xa inhibitors (DiXaIs), in human citrated plasma, using a manual or automated method. This method is appropriate for the Apixaban, Rivaroxaban and Edoxaban assay, direct Factor Xa (FXa) inhibitors.
This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:

Technical:
The BIOPHEN™ DiXaI kit is a two stages chromogenic method specific to FXa direct inhibitors and insensitive to heparins (UFH and LMWH).

PRINCIPLE:

BIOPHEN™ DiXaI is a chromogenic method based on the inhibition, by the DiXaI being assayed, of a constant and excess quantity of Factor Xa (FXa). The residual Factor Xa hydrolyses the FXa-specific chromogenic substrate, releasing paranitroaniline (pNa). The amount of pNa released (measured by absorbance at 405 nm) is inversely proportional to the concentration of DiXaI in the sample.

REAGENTS:

R1 **FXa (h):** Purified human Factor Xa at approximately 10 U/mL, lyophilized. Contains BSA and stabilizers.
R2 **Factor Xa-specific chromogenic substrate (CS-11(65))** at approximately 2 mg/mL, lyophilized. Contains stabilizers.
R3 **Tris-NaCl-EDTA reaction buffer,** pH 7.85, liquid form. Contains a heparin neutralizing substance. Contains preservatives and stabilizers.

The product is classified as non-hazardous and is not subject to labeling according to EC Regulation No. 1272/2008 [CLP].

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.
- Use only the reagents from the same batch of kits.
- Waste should be disposed of in accordance with applicable local regulations.
- This device of *in vitro* use is intended for professional use in the laboratory.

REAGENT PREPARATION:

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

R1 R2 Reconstitute the contents of each vial with exactly **2.5 mL of distilled water.**
Shake vigorously until complete dissolution 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.

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

- **14 days** 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:

Reagents:

- Specific calibrators and controls:

Calibrators	**BIOPHEN™ Apixaban Calibrator / Calibrator Low**	**BIOPHEN™ Rivaroxaban Plasma Calibrator / Calibrator Low**	**BIOPHEN™ Edoxaban Calibrator / Calibrator Low**
References	226201-RUO / 226101-RUO	222701-RUO / 226001-RUO	226501-RUO / 226401-RUO
Controls	**BIOPHEN™ Apixaban Control / Control Low**	**BIOPHEN™ Rivaroxaban Control Plasma / Control Low**	**BIOPHEN™ Edoxaban Control / Control Low**
References	225301-RUO / 225201-RUO	224501-RUO / 225101-RUO	225501-RUO / 225401-RUO

- Automatic analyzer for chromogenic assays such as: CS-series, STA-R® family, ACL-TOP® family, CN-series.
- Laboratory material.
- When required, Tris-NaCl-EDTA buffer (AR032A-RUO / AR032K-RUO).

Materials (endpoint method):

- 20% acetic acid or 2% citric acid
- Spectrophotometer and chromogenic assay analyzer.
- Water-bath.
- Stopwatch, calibrated pipettes.

Please note that the applications on other analyzers can be validated by the instrument manufacturer under their responsibility.

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[1-2].
The blood (9 volumes) should be carefully collected onto the trisodium citrate anticoagulant (1 volume) (0.109 M, 3.2%) by clean venipuncture.
CLSI H21-A5[1] and studies[2]:

- 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:

The kit can be used for kinetics, automated or manual (endpoint) methods. Perform the test at **37°C** and read color intensity at **405nm.**

Automated method:
For an automated method, application guides are available on request. See specific Application Guide and specific precautions for each analyzer.

Assay method:
Rivaroxaban assay:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. Calibrators should be diluted in the R3 buffer as described in the table below in order to prepare the calibration curve:
2. Dilute the specimens and controls in R3 buffer, as described in the table below:

Dosage	Calibrators Reference	Controls reference	Dilution in reagent R3
Rivaroxaban	222701-RUO	224501-RUO	**1/15**
Rivaroxaban low range	226001-RUO	225101-RUO	**1/3**
Samples	NA	NA	**1/15 (standard range) 1/3 (low range)**

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. Add the following to a plastic tube incubated at **37°C**:

Reagents	Volume
Calibrators, or test plasmas, or controls diluted **in** R3	200 µL
R1 **FXa (h)** pre-incubated at **37°C**	200 µL
Mix and incubate **at 37°C for exactly 1 minute**, then add the following:	
R2 **Substrate** pre-incubated **at 37°C**	200 µL
Mix and incubate at **37°C,** for **45 seconds exactly**	
Stop the reaction by adding:	
Citric acid (2%)*	400 µL
Mix and measure the optical density at **405 nm** against the corresponding blank.	

*Or acetic acid (20%). The resulting yellow colour is stable for 2 hours.
The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Acetic acid (20%) or citric acid (2%), substrate, Factor Xa(h), diluted test sample.

Measure the optical density at 405 nm. Subtract the measured blank value from the test absorbance.

Apixaban assay:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. Calibrators should be diluted in the R3 buffer as described in the table below in order to prepare the calibration curve:
2. Dilute the specimens and controls in R3 buffer, as described in the table below:

Dosage	Calibrators reference	Controls reference	Dilution in reagent R3
Apixaban	226201-RUO	225301-RUO	**1/40**
Apixaban low range	226101-RUO	225201-RUO	**1/6**
Samples	NA	NA	**1/40 (standard range) 1/6 (low range)**

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. Add the following to a plastic tube incubated at **37°C**:

Reagents	Volume
Calibrators, or test plasmas, or controls diluted **in** R3	200 µL
R1 **FXa (h)** pre-incubated at **37°C**	200 µL
Mix and incubate **at 37°C for exactly 1 minute**, then add the following:	
R2 **Substrate** pre-incubated **at 37°C**	200 µL
Mix and incubate at **37°C,** for **45 seconds exactly**	
Stop the reaction by adding:	
Citric acid (2%)*	400 µL
Mix and measure the optical density at **405 nm** against the corresponding blank.	

*Or acetic acid (20%). The resulting yellow colour is stable for 2 hours.

The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Acetic acid (20%) or citric acid (2%), substrate, Factor Xa(h), diluted test sample.

Measure the optical density at 405 nm. Subtract the measured blank value from the test absorbance.

Edoxaban assay:

1. Reconstitute the calibrators and controls as indicated in the specific instructions. Calibrators should be diluted in the R3 buffer as described in the table below in order to prepare the calibration curve:
2. Dilute the specimens and controls in R3 buffer, as described in the table below:

Dosage	Calibrators reference	Controls reference	Dilution in reagent R3
Edoxaban	226501-RUO	225501-RUO	**1/15**
Edoxaban low range	226401-RUO	225401-RUO	**1/4**
Samples	NA	NA	**1/15 (standard range) 1/4 (low range)**

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. Add the following to a plastic tube incubated at **37°C**:

Reagents	Volume
Calibrators, or test plasmas, or controls diluted **in** R3	200 µL
R1 **FXa (h)** pre-incubated at **37°C**	200 µL
Mix and incubate **at 37°C for exactly 1 minute**, then add the following:	
R2 **Substrate** pre-incubated **at 37°C**	200 µL
Mix and incubate at **37°C,** for **45 seconds exactly**	
Stop the reaction by adding:	
Citric acid (2%)*	400 µL
Mix and measure the optical density at **405 nm** against the corresponding blank.	

*Or acetic acid (20%). The resulting yellow colour is stable for 2 hours.

The specimen blank is obtained by mixing the reagents in the reverse order to that of the test: Acetic acid (20%) or citric acid (2%), substrate, Factor Xa(h), diluted test sample.

Measure the optical density at 405 nm. Subtract the measured blank value from the test absorbance.

Create a plasma blank if specimen is icteric, lipaemic, haemolysed, or if its color differs from the standard plasmas.

When employing the kinetic method, use ΔOD 405 instead of OD 405. In this case, it is not necessary to subtract the specimen blank, or to stop the reaction.

If a reaction volume other than that indicated above is required for the method used, the volume ratio must be strictly observed in order to guarantee assay performance. The user is responsible for validating any changes and their impact on all results.

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 defined, preferably for each test series, and at least for each new reagent batch, or after analyser maintenance, or when the measured quality control values fall outside the acceptable range for the method.

Each laboratory must define its acceptable ranges and verify the expected performance in its analytical system.

RESULTS:

- For the manual endpoint method, plot the calibration curve, with the OD 405 nm along the Y-axis and the analyte concentration, expressed as (ng/mL), along the X-axis:
  - **Rivaroxaban low range**, use a **Lin-Log** scale (ng/mL – OD).
  - **Rivaroxaban standard range,** use a **Lin-Lin** scale (ng/mL – OD).
  - **Apixaban**, use a **Lin-Lin** scale (ng/mL – OD) for both ranges.
  - **Edoxaban low range**, use a **Lin-Lin** scale (ng/mL – OD).
  - **Edoxaban standard range**, use a **Lin-Log** scale (ng/mL – OD).
- When employing the kinetic method, use ΔOD 405 instead of OD 405.
- The concentration of DiXal (ng/mL) in the test specimen is directly inferred from the calibration curve, when the standard dilution is used.

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.
- Highly concentrated samples can be pre-diluted in a pool of normal plasmas. The measured concentrations should then be multiplied by the supplementary dilution factor.
- 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.
- Anti-FXa activity levels may be overestimated in samples containing specific Factor Xa reversal agents, such as andexanet alfa.

PERFORMANCES:

Performances studies were conducted on analyzers.

The following performance data represent typical results and are not to be regarded as specifications for BIOPHEN™ DiXaI.

Mathematical analyses are performed using a validated statistical software built in accordance with state of the art.

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.

Precision

Precision studies were assessed using laboratory controls and spiked pooled plasmas. Coefficient of variation (CV) for all samples is less than 8.0% for repeatability, less than 10.0% for reproducibility and less than 10.0% 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.

By the assay principle, no coagulation factor interference, such as Factor II and X, is expected.

The assay is completely insensitive to heparins (UFH and LMWH) up to 2 IU/mL

REFERENCES:

1. CLSI Document H21-A5: “Collection, transport, and processing of blood specimens for testing plasma -based coagulation assays and molecular hemostasis assays; approved guideline” (2008).
2. Gosselin R.C. *et al*. International Council for Standardization in Haematology (ICSH) Recommendations for Laboratory Measurement of Direct Oral Anticoagulants. Thromb Haemost. 2018.

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:

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
UDI	Unique Device Identifier	BIO	Contains biological material of animal origin		Contains human blood or plasma derivatives
DANGER	Danger	WARNING	Attention	ACCEPTANCE RANGE	Acceptance range

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155 rue d'Eragny, 95000 Neuville-sur-Oise, France