BIOPHEN™ ANTI-Xa
(2 Stages Heparin Assay)
Ref 221005
(R1, R2, R3 : 2 x 1 mL)

Two stages Anti-Xa chromogenic method for the Heparin activity measurement on plasma or purified medium, according to Pharmacopoeia (USP, EP).

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

INTENDED USE:
This BIOPHEN™ ANTI-Xa (2 Stages Heparin Assay) kit is a two-stage chromogenic assay for measuring the activity of heparins (UFH or LMWH), in manual or automatic methods. This method is proposed only to test heparin in human citrated plasma, or in purified solution. This kit is for research use only and must not be used for patient diagnosis or treatment.

SUMMARY AND EXPLANATION:
Heparin is a sulphated polysaccharide with a high affinity for antithrombin. Complexed with heparin, antithrombin exhibits a fast acting and potent inhibitory activity for coagulant serine esterases: Xa, Xa and thrombin. Low Molecular Weight Heparin (LMWH), and analogues such as Sodium Danaparoid, inhibit more efficiently Factor Xa than thrombin, whereas the Unfractionated Heparin (UFH) inhibit more efficiently thrombin than other serine esterases. The Pentasaccharide (Arixtra™) inhibits more specifically Factor Xa. This heparin assay is a two-stage assay for measuring accurately and sensitively heparin concentrations in plasma or in purified systems. Tested plasma needs to be diluted before assaying it.

This assay, using a predilution of Antithrombin and Factor Xa reagents in specific buffer (not included within this kit), is in compliance with the United States Pharmacopoeia (USP) and European Pharmacopoeia (EP).

PRINCIPLE:
The BIOPHEN™ ANTI-Xa (2 Stages Heparin Assay) kit is a chromogenic anti-Xa method, developed for measuring Unfractionated Heparins (UFH) and Low Molecular Weight Heparins (LMWH) in plasma or in purified solutions, for their Anti-Xa activity. The BIOPHEN™ ANTI-Xa (2 Stages Heparin Assay) assay is a method based on the inhibition of a constant amount of Factor Xa (Fxa), by the tested heparin in presence of exogenous antithrombin (stage 1), and hydrolysis of a Factor Xa specific chromogenic substrate (CS11(65)), by the Factor Xa in excess (stage 2). pNA is then released from the substrate. The amount of pNA released (measured at 405 nm) is then a relation of the residual Factor Xa activity. There is an inverse relationship between the concentration of heparin and color development.

Heparin + AT → [AT Hesp.]
[AT Hesp.] + [Fxa (excess)] → [Fxa-AT-Hesp.]+ [residual Fxa] → [residual Fxa] + Substrate → Peptide + pNA

REAGENTS:
R1: Reagent 1: ATIII (h)
Human Antithrombin (ATIII), lyophilized vial containing about 5 IL/mL. Contains BSA. 2 vials of 1 mL.

R2: Reagent 2: Fxa (b)
Purified bovine Factor Xa, lyophilized vial containing about 40 µg (i.e. about 90 nkat, when determined in optimized conditions with CS11(65)), by the Factor Xa in excess (stage 2). The stability of the thawed reagent should be checked under laboratory work conditions.

R3: Reagent 3: Factor Xa specific chromogenic substrate
Chromogenic substrate specific for Factor Xa (CS11(65)), vial of about 4 mg (about 6 µmol), lyophilized in presence of mannitol. 2 vials of 1 mL.

WARNINGS AND PRECAUTIONS:
- Biological products must be handled with all necessary precautions and considered as being potentially infectious.
- A yellow color indicates a contaminated substrate. Discard the vial and use a new one.
- Waste should be disposed of in accordance with applicable local regulations.
- Use only the reagents from the same batch of kits. Do not mix reagents from different kit batches when performing an assay; they are optimized for each batch of kits.
- Handle the reagents with care to avoid contamination during use. If possible, avoid reagent evaporation during use by limiting the liquid-air exchange surface. Evaporation reduces the reagent’s stability in the analyzer.

A plasma sample used to prepare the human antithrombin has been tested by recorded methods and is certified free of HIV antibodies, Hbs Antigen and HCV antibodies. The bovine plasma used to prepare the BSA and Factor Xa has been tested by recorded methods and is certified free of infectious agents, in particular the causative agent of bovine spongiform encephalitis.

To preserve reagent stability, seal the vials after use with their respective caps.

When employing the kinetic method, use AOD 405 instead of OD 405.

The Factor Xa and AT concentrations are adjusted if required for each lot for providing the right reactivity in the assay.

Storage conditions: 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.

REAGENT PREPARATION AND STABILITY:
The reagents are lyophilized under a vacuum in their vials. To avoid any product loss when opening the vial of lyophilized reagents, gently remove the freeze-drying stopper.

R1: Reagent 1: ATIII (h)
Reconstitute the contents of each vial with exactly 1 mL distilled water, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.

Just before use, dilute 1/5 in the appropriate buffer according to the Heparin to be assayed (see table below, if the whole vial is used, add 4 mL of buffer to the 1 mL of restored ATIII).

Homogenize the reagent prior to use.

R2: Reagent 2: Fxa (b)
Reconstitute the contents of each vial with exactly 1 mL distilled water, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.

Just before use, dilute 1/5 in the appropriate buffer according to the Heparin to be assayed (see table below, if the whole vial is used, add 4 mL of buffer to the 1 mL of restored Factor Xa). Homogenize the reagent prior to use.

R3: Reagent 3: Factor Xa specific chromogenic substrate
Reconstitute the contents of each vial with exactly 1 mL distilled water, shake vigorously until fully dissolved. Allow to stabilize for 30 min. at room temperature (18-25°C), shaking occasionally.

Just before use, dilute 1/5 in the appropriate buffer according to the Heparin to be assayed (see table below, if the whole vial is used, add 4 mL of buffer to the 1 mL of restored Factor Xa).

Homogenize the reagent prior to use.

Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is of:
- 15 days at 2-8°C.
- 7 days at room temperature (18-25°C).
- 2 months frozen at -20°C or less

Storage conditions:
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.

REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED:
Reagents:
- Distilled water.
- 20% acetic acid or 2% citric acid (and point method).
- Specific buffers such as:

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<tr>
<th>Product Name</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Tris-EDTA-HC5-PES, pH 8.40</td>
<td>AR003K</td>
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<tr>
<td>Tris-EDTA-BSA, pH 7.40</td>
<td>AR005L</td>
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<tr>
<td>Tris-NaCl, pH 7.40</td>
<td>AR002K</td>
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<tr>
<td>Tris-EDTA-NaCl, pH 8.40</td>
<td>AR029K</td>
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<tr>
<th>Product Name</th>
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<tr>
<td>BIOPHEN UFH Control Plasma</td>
<td>223010-ROU</td>
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<tr>
<td>BIOPHEN LMWH Control Plasma</td>
<td>223020-ROU</td>
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<td>BIOPHEN Heparin Calibrator</td>
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<td>BIOPHEN UFH Calibrator</td>
<td>222030-ROU</td>
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**SPECIFIC COLLECTION AND PREPARATION:**

Specimens must be collected and stored according to applicable local guidelines.

**Materials:**
- Spectrophotometer or automatic instrument for chromogenic assays.
- Stopwatch: Calibrated pipettes; Plastic tubes or microplate.

**Collection:**
- Human plasma obtained from anticoagulated blood (trisodium citrate).

**Methods:**
- Can be automated using the BIOPHEN® Anti-Xa (2 Stages Heparin Assay) assay. The assay can be calibrated for the assay of LMWH, UFH and their analogs. Calibrators and controls specific kit covering the dynamic test range is available from HYPHEN BioMed (see the "REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED" paragraph) and can be used to establish the calibration curve.

**Automated tests:**
- Can be used for kinetic, automated or manual (endpoint) methods. Perform the test at 37°C and read color intensity at 405nm.

**Materials:**
- BIOPHEN LMWH Control Low
- BIOPHEN® Anti-Xa (2 Stages Heparin Assay) - LMWH solution
- BIOPHEN® Anti-Xa (2 Stages Heparin Assay) - UFH solution
- BOVINE LMWH Control Low
- Citric acid (2%)*

**Sample dilution and incubation times:**
- LMWH: 1 hour at room temperature (18-25°C).
- UFH: 4 hours at room temperature (18-25°C).
- Frozen plasma specimens should be thawed rapidly at 37°C, then shaken thoroughly and tested immediately. Resuspend any precipitate by shaking vigorously immediately after thawing and before use.

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

**Automated methods:**
- Applications for the various analyzers are available on request. See the specific application and specific precautions for each analyzer.

**Limitations:**
- To ensure optimum test performance and to meet the specifications, the technical instructions validated by HYPHEN BioMed should be followed carefully.

**RESULTS:**
- 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 analyzer maintenance, or when the measured quality control values fail outside the acceptable range for the method.

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

**REFERENCES:**
- Fillet and others, Applications for the various analyzers are available on request. See the specific application and specific precautions for each analyzer.

**QUALITY CONTROL:**
- 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 analyzer maintenance, or when the measured quality control values fail outside the acceptable range for the method.

**LIMITATIONS:**
- 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 analyzer maintenance, or when the measured quality control values fail outside the acceptable range for the method.

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

**REFERENCES:**

**SYMBOLS:**
- Symbols used and signs listed in the ISO 15223-1 standard, see Symbol definitions document.

**Changes compared to the previous version:**

**CALIBRATION:**
- The BIOPHEN™ Anti-Xa (2 Stages Heparin Assay) assay can be calibrated for the assay of LMWH, UFH and their analogs. Calibrators and controls specific kit covering the dynamic test range is available from HYPHEN BioMed (see the "REAGENTS AND MATERIALS REQUIRED BUT NOT PROVIDED" paragraph) and can be used to establish the calibration curve.

The calibration curves on CS-series shown below are given by way of example only. The calibration curve established for the assay series must be used.