5-TEST USP-UFH Anti-Xa starter set in compliance with US Pharmacopoeia

REF 5D-90458

Complete set of individual reagents for the measurement of heparin and heparin-like anticoagulants in aqueous solutions using an anti-FXa chromogenic assay for pharmaceutical preparations in compliance with US Pharmacopoeia.

For Research Use Only.
Not for Use in Diagnostic Procedures.
Mixed storage.

INTENDED USE

This Heparin Anti-FXa method can be used as an endpoint or kinetic chromogenic assay for measuring the concentration of heparin and heparin-like anticoagulants in heparin concentration ranges from 0.03-0.375 USP Heparin Units/mL (IU/mL). This method is to be used for the determination of anti-FXa activity of Unfractionated Heparin following the recommendations of the US Pharmacopoeia.

TEST PRINCIPLE

Heparin is a sulphated polysaccharide with a high affinity for antithrombin. Antithrombin complexed with heparin has a fast and potent inhibitory activity for coagulation factors IXa, Xa and IIa (Thrombin). FXa in excess, is neutralized in proportion to the amount of heparin (Heparin · AT- complex). The remaining amount of FXa hydrolyses the chromogenic substrate and liberates the chromophoric group pNA. The colour is then read photometrically at 405 nm. There is an inverse relationship between the concentration of heparin and colour development measured at 405 nm.

Heparin + AT → [AT Hep.]

[AT Hep.] + [FXa (excess)] → [FXa-AT-Hep.] + [residual FXa]

[residual FXa] + Substrate → Peptide + pNA

REAGENTS INCLUDED

5-BUFFER USP/Ph.Eur. Tris-NaCl-EDTA-PEG-6000 Buffer salts
Ref. 5D-80434
5-BUFFER USP Tris-NaCl-PEG-6000 Buffer salts pH 7.4
0.050 M Tris buffer pH 8.4 at 25°C, 0.175 M NaCl, 0.0075 M EDTA, 0.1% (w/v) PEG-6000
Kit content: 1 Pouch
Reconstitution: dissolve pouch content in 1000 mL distilled water.
Buffer stability after reconstitution: 4 weeks at 2-8°C when protected from any contamination.

5-ENZYME Factor Xa (Bovine)
Ref. 5D-60217
Lyophilized Bovine FXa
Kit content: 3 Vials, 30 µg per vial
Reconstitution: dissolve vial content in 2 mL distilled water
Stock concentration: 15 µg/mL
Working concentration: 2.5 µg/mL (stock solution diluted 1:6 in 5-BUFFER 5D-80434). Concentration may be adopted as requested.
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is:

- 15 days at 2-8°C.
- 4 days at room temperature (18-25°C).
- 6 months frozen at -20°C or less*

5-PROTEIN Antithrombin (Human)
Ref. 5D-60104
Lyophilized Human Antithrombin III
Kit content: 2 Vials, 10 IU per vial
Reconstitution: dissolve vial content in 2 mL distilled water
Stock concentration: 5 IU/mL
Working concentration: 1 IU/mL (stock solution diluted 1:5 in 5-BUFFER 5D-80434)
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is:

- 15 days at 2-8°C.
- 4 days at room temperature (18-25°C).
- 6 months frozen at -20°C or less*

5-CHROM-65 Chromogenic Factor Xa Substrate
Ref. 5D-30807
Lyophilized Chromogenic Substrate for Factor Xa: Z-D-Arg-Gly-Arg-pNA·2HCl
Kit content: 1 Vial with 25 mg (39 µmol/vial) synthetic chromogenic Factor Xa Substrate, highly purified and stabilized. Mannitol is added as a bulking agent.
Reconstitution: dissolve vial content in 7.8 mL water
Stock concentration: 5 mM
Working concentration: 1 mM (stock solution diluted 1:5 in distilled water)
Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is:

- 15 days at 2-8°C.
- 4 days at room temperature (18-25°C).
- 6 months frozen at -20°C or less*

STORAGE CONDITIONS:

Unopened reagents must be stored in their original packaging at 2–8°C. They are then stable until the expiration date printed on the label.
Stability of diluted reagents should be checked in the working conditions of the laboratory user.

*Thaw only once, as rapidly as possible at 37°C, adapting the incubation period to the volume of reagent. The stability of the thawed reagent should be checked under laboratory work conditions.

OTHER REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water
- Acetic acid 20 % V/V
- USP, EP or International Standards from NIBSC, Internal Reference preparations

Materials:

- Spectrophotometer or automatic instrument for chromogenic assays
- Stopwatch
- Calibrated pipettes
- Water bath or heating block
- Plastic tubes or 96 well microplates

TEST PROCEDURE

Prepare at least 5 dilutions of your reference Heparin Preparation spanning the concentration range from 0.03 to 0.375 USP Heparin Units/mL (IU/mL) in pH 8.4 Buffer.

Prepare 5 dilutions of your sample in 5-BUFFER 5D-80434 to have approximately the same activity equal to those of the Standard solution .

Perform the test with each Standard and Sample dilution in duplicate in suitable plastic tubes in a water bath or heating block set at 37°C.

Add to each tube 120 µL of 5-BUFFER 5D-80434 and then separately add 30 µL of the different dilutions of refence and samples.

Add 150 µL of preheated Antithrombin III solution to each tube , mix gently and incubate 120 seconds at 37°C.

Add 300 µL of preheated Bovine Factor Xa solution and incubate 120 seconds at 37°C.

Add 300 µL of preheated FXa Chromogenic Substrate solution and incubate for 120 seconds at 37°C. If necessary, adjust the incubation time to give best dose-response curve.

Stop the reaction with 150 µL acetic acid solution.

Prepare a Blank for zeroing the spectrophotometer by adding the reagents in reverse order starting with the acetic acid and ending with 150 µL of 5-BUFFER 5D-80434.

Measure the absorbance at 405 nm.

Plot the log of the absorbance versus heparin concentrations in USP Heparin Units/mL (IU/mL). Determine the slope for the regression line of both reference and sample curves to calculate the potency.

Follow statistical analysis of results of biological assays and tests in compliance with US Pharmacopoeia guidelines for slope ratio assays.

The anti-Xa activity of the sample is calculated with the formula:

Result = A x (Slope Sample Curve/Slope Reference Curve)

Where A is the potency of the Refence preparation used.

Test Tube Method

Reagent	Volume
Buffer	120 µL
Diluted Reference or Sample	30 µL
Antithrombin III 1 U/mL preheated at 37°C	150 µL
Mix and incubate for 2 minutes at 37°C	
Bovine Factor Xa 2.5 µg/mL preheated at 37°C	300 µL
Mix and incubate for 2 minutes at 37°C	
Chromogenic substrate 1 mM preheated at 37°C	300 µL
Mix and incubate for 2 minutes at 37°C Stop the reaction by adding:	
Acetic acid 20%	150 µL
Mix and measure the absorbance at 405 nm	

ALTERNATIVE METHODS

The assay can be miniaturized in 96 wells microplate.

Dilute the prepared reference and sample dilutions further 1:5 with Buffer. For example, add 120 µL of 5-BUFFER 5D-80434 to 30 µL of these dilutions.

Microplate Method

Reagent	Volume
1:5 Diluted Reference or Sample dilutions	40 µL
Antithrombin III 1 U/mL preheated at 37°C	40 µL
Mix and incubate for 2 minutes at 37°C	
Bovine Factor Xa 2.5 µg/mL preheated at 37°C	80 µL
Mix and incubate for 2 minutes at 37°C	
Chromogenic substrate 1 mM preheated at 37°C	80 µL
Mix and incubate for 2 minutes at 37°C Stop the reaction by adding:	
Acetic acid 20%	20 µL
Mix and measure the absorbance at 405 nm	

Application protocols for automated analysers are available from info@5-diagnostics.com.

ASSAY DETECTION RANGE

0.03-0.375 USP Heparin Units/mL (IU/mL)

APPLICATIONS

Measurement of the specific anti-FXa activity of heparin and heparin-like anticoagulants in purified milieu using a two-stage assay. This procedure is in compliance with the quality control of Unfractionated Heparin preparations listed in US Pharmacopoeia.

REFERENCES

USP 40(208) Anti-Factor Xa and Anti-Factor IIa Assays for Unfractionated and Low Molecular Weight Heparins

5-DIAGNOSTICS

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