

5-TEST EP-UFH Anti-Xa starter set in compliance with European Pharmacopoeia

REF 5D-90454

Complete set of individual reagents for the measurement of heparin in aqueous solutions using an anti-FXa chromogenic assay for pharmaceutical preparations in compliance with Eur. 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 in the range from 0.03-0.375 IU/mL. This method is to be used for the determination of anti-FXa activity of Unfractionated Heparin following the recommendations of the European 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
pH 8.4**

Ref. 5D-80434

5-BUFFER USP/Ph.Eur. Tris-NaCl-EDTA-PEG-6000 Buffer salts pH 8.4
0.050 M Tris Buffer pH 8.4 at 25°C, 0.175 M NaCl, 0.0075 M EDTA,
0.10% /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: 3 µg/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:

- 3 months at 2-8°C.
- 7 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 Reagent

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 Buffer 5D-80434)

Reagent stability after reconstitution, excluding any contamination or evaporation, and stored in the original vial, is:

- 1 month at 2-8°C.
- 72 hours at room temperature (18-25°C).
- 6 months frozen at -20°C or less.*

5-CHROM Chromogenic Factor Xa Substrate

Ref. 5D-30807

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 distilled 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:

- 3 months at 2-8°C.
- 7 days at room temperature (18-25°C).
- Do not freeze.

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.

OTHER REAGENTS AND MATERIAL REQUIRED BUT NOT PROVIDED:

Reagents:

- Distilled water
- Glacial 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 4 independent calibration curves of minimum 4 points spanning 0.03 to 0.375 IU/mL of your reference Heparin Preparation in 5-BUFFER 5D-80434. Use 5-BUFFER 5D-80434 as a blank for the reaction.

Prepare 4 independent dilutions of your sample in 5-BUFFER 5D-80434.

Add 50 µL of 5-PROTEIN Antithrombin (Human) solution to 50 µL of sample or calibrator or blank. Mix gently and incubate 60 seconds at 37°C in a water bath or heating block.

Add 100 µL of 5-ENZYME Factor Xa (Bovine) solution and incubate 120 seconds at 37°C.

Add 100 µL of pre-warmed 5-CHROM Chromogenic Factor Xa Substrate solution and incubate exactly for 240 seconds at 37°C.

Stop the reaction with 20 µL 20% acetic acid solution.

Measure the absorbance at 405 nm.

Plot the absorbance versus log of heparin concentrations in International Units/mL.

If necessary adjust the incubation time to give best dose-response curve.

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 Pharmacopoeia guidelines for parallel-line assays.

Reagent	Tube
Antithrombin III 1 IU/mL	50 µL
Reference, test sample or blank	50 µL
Mix and incubate for 1 minute at 37°C	
Bovine Factor Xa 3ug/mL	100 µL
Mix and incubate for 2 minutes at 37°C	
Chromogenic substrate 1 mM pre-warmed at 37°C	100 µL
Mix and incubate at 37°C exactly for 4 minutes Stop the reaction by adding:	
Acetic acid 20%	20 µL
Mix and measure the absorbance at 405nm against the corresponding blank.	

ALTERNATIVE METHODS

The assay can be miniaturized in 96 wells microplate.

Reagent	Microplate
Antithrombin III 1 IU/mL	30 µL
Reference, test sample or blank	30 µL
Mix and incubate for 1 minute at 37°C	
Bovine Factor Xa 3ug/mL	60 µL
Mix and incubate for 2 minutes at 37°C	
Chromogenic substrate 1 mM pre-warmed at 37°C	60 µL
Mix and incubate at 37°C exactly for 4 minutes Stop the reaction by adding:	
Acetic acid 20%	20 µL
Mix and measure the absorbance at 405nm against the corresponding blank.	

Note: Reagent concentrations may be adapted in order to obtain higher OD values. Please contact info@5-diagnostics.com.

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

ASSAY DETECTION RANGE:

0.03-0.375 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 Heparin preparations listed in European Pharmacopoeia.

REFERENCES:

European Pharmacopoeia 8.0: 01/2015:20705 Assay of Heparin



5-Diagnostics AG
Heuberg 7, 4051 Basel,
Switzerland
Tel: +41 61 588 07 84
www.5-diagnostics.com
info@5-diagnostics.com