Enoxaparin Factor Xa ELISA Kit

Cat #: orb608258 (manual)

Chromogenic assay for testing Enoxaparin in purified systems by measurement of factor Xa inhibition, in compliance with pharmacopoeias (EP/BP) and FDA guidelines

For Research & Industrial Purposes Only. ***NOT*** *for use in diagnostic or therapeutic procedures. Purchase does not include or carry the right to resell or transfer this product either as a stand-alone product or as a component of another product.*

Intended Use:

Enoxaparin Factor Xa is a chromogenic assay intended for the quantitative determination of enoxaparin in purified solutions by measurement of factor Xa inhibition activity. The kit can be used for 100 test reactions as per microtiter plate protocol.

Principle:

The inhibitory effect of anti-thrombin III (AT-III) on thrombin, factor Xa and other coagulation serine proteases in plasma is increased several thousand-fold by enoxaparin. This inhibition accounts for the anticoagulant effect of enoxaparin. The quantitative determination of enoxaparin levels by the measurement of their anti-Xa activity is a necessary tool for monitoring treatment efficacy.

Presence of Enoxaparin catalyzes the reaction between AT-lll and Factor Xa. The factor Xa inhibition test is the most useful assay covering the widest variety of enoxaparin preparations. In the assay, the rate of factor Xa inhibition is directly proportional to the enoxaparin concentration since both factor Xa and AT-III are in excess. The residual factor Xa activity is inversely proportional to the enoxaparin concentration.

Materials Provided:

1. Human Anti-thrombin III Reagent (lyophilized) - 1 vial
2. Bovine Factor Xa Reagent (lyophilized) - 1 vial
3. Chromogenic Substrate (lyophilized) - 2 vials
4. Instruction Manual

Materials to be provided by the End-User:

1. Microplate Reader / Spectrophotometer able to measure absorbance at 405nm
2. Adjustable pipettes to measure volumes ranging from 25µl to 2500µl, duly calibrated
3. Deionized (DI) water
4. Parallel line software for data analysis

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5. Plastic tubes or cuvettes or microtiter plates with overflow capacity ≤ 350µl/well
6. 37°C water bath or dry bath
7. Timer/Stop watch
8. Glacial Acetic Acid
9. Absorbent paper
10. Dilution Buffer
11. Standard

Storage and Stability Information:

Unreconstituted (lyophilized) reagents are stable until the expiration date indicated on the label when stored at 2 to 8°C.

1. **Human Anti-thrombin III Reagent:** Reconstituted reagent is stable for 2 weeks at 2° to 8° C and for 4 months at -20°C.
2. **Bovine Factor Xa Reagent:** Reconstituted reagent is stable for 2 weeks at 2° to 8° C and for 4 months at -20°C.
3. **Chromogenic Substrate:** Reconstituted reagent is stable for 2 weeks at 2° to 8° C and for 4 months at -20°C.
4. **Dilution Buffer** and **Acetic acid** are to be freshly prepared, prior to use.

Health Hazard Warnings:

1. The source material for the human anti-thrombin III has been found to be non-reactive for Hepatitis B Surface Antigen (HBsAg), Hepatitis C Virus (HCV) and Human Immunodeficiency Virus Type 1 and Type 2 (HIV-1, HIV- 2) using FDA approved methods.
2. The enoxaparin (anti-FXa) anti-thrombin III reagent contains sodium azide that may react with lead or copper plumbing to form highly explosive azides.

Specimen Collection and Handling:

Purified Samples: Dilute the enoxaparin preparation with Dilution Buffer in order to bring it at a concentration within the assay working range.

Reagent Preparation:

Note:

1) Bring all reagents to room temperature.

2) All reagents should be diluted immediately prior to use.

1. **Human Anti-thrombin III Reagent:**

   Anti-thrombin III is a lyophilized preparation. For Reconstitution, add 5 ml of Distilled water and leave for 15 minutes before running the experiment, prepare working solution by adding 1ml of reconstituted HAT- III reagent with 11 ml of Distilled water. Use this working solution.

2. **Bovine Factor Xa Reagent:**

   Factor Xa Reagent is a lyophilized preparation. For Reconstitution, add 7.5ml of Distilled water and leave it to stand for 15 minutes.

3. **Chromogenic Substrate:**

   Chromogenic Substrate is a lyophilized substrate specific for Factor Xa activity. For Reconstitution, add 5 ml of Distilled water and leave it to stand for 15 minutes.

4. **Dilution Buffer: For Standard / Sample** (Not provided in the kit):

   To be prepared with 50mM Tris, 150mM Nacl Adjust the pH upto 7.4

5. **Acetic Acid Solution(Stop Solution):** (Not provided in the kit):

   20% v/v Glacial Acetic Acid: 20 ml of Glacial Acetic Acid in 80 ml of Distilled Water.

6. **Standard and Test Concentration:** Recommended range of standard and Test concentration includes: 0.10 IU/ml, 0.075 IU/ml, 0.050 IU/ml, and 0.025IU/ml.

For Example:

Preparation of Standard Concentrations

Standard Concentration 500 IU/mL (Main Stock) is to be diluted as per below table:

Standard Dilution

Sr No.	Concentration (IU/ml)	Stock (µL)	Diluent Buffer pH 7.4 (µL)	Total Volume (µL)
S1	50	50ul of M.S	450	500
S2	1	20ul of S1	980	1000
S3	0.10	30ul of S2	270	300
S4	0.075	22.5ul of S2	275.5	300
S5	0.050	300ul of S3	300	600
S6	0.025	150ul of S5	150	300

Test Dilution – Test Sample Main Stock is of concentration 500IU/mL

Sr No.	Concentration (IU/ml)	Stock (µL)	Diluent Buffer pH 7.4 (µL)	Total Volume (µL)
T1	50	50ul of M.S	450	500
T2	1	20ul of T1	980	1000
T3	0.10	30ul of T2	270	300
T4	0.075	22.5ul of T2	275.5	300
T5	0.050	300ul of T3	300	600
T6	0.025	150ul of T5	150	300

Assay Protocol:

Add the reagents into the microwell as per following steps:

	microwell
Anti-thrombin III	20µl
Standard or Test Sample	20µl
Mix but do not allow bubbles to form. Incubate at 37ºC, for 1 minute	
Bovine Factor Xa	50µl
Mix and incubate at 37ºC, for exactly 1 minute	
Chromogenic Substrate	100µl
Mix and incubate at 37ºC, for 4 minutes	
Acetic Acid	100µl
Mix and measure the absorbance at 405nm	

Calculation of Results:

For each series, calculate the regression of the absorbance against log concentration of the sample solutions and the standard solutions. Calculate the potency of the enoxaparin in IU of Anti-Factor Xa activity/ml using statistical methods for parallel-line assays. The four independent log relative potency estimates are then combined to obtain the final geometric mean. Its confidence limits are calculated. Express the Anti-Factor Xa activity of the sample in mg.

Standard and Test Samples being serial diluted should pass the test for linearity and parallelism as the interpretation is done by extrapolating the data. We have used proprietary MS Excel software for the same based on DJ Finney algorithm.

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