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KRISHZYME™

Enoxaparin Factor IIa Assay kit

REF: KBBA03ES

Ver4.4

(100 test)

RUO

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

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	Store At	**LOT**	**Batch Code**
	Manufactured By		**Biological Risk**
	Expiry Date		**Consult Operating Instructions**

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. Any use of this product other than the permitted use without the express written authorization of KRISHGEN BioSystems is strictly prohibited.*

Intended Use:

Enoxaparin Factor lla is a chromogenic assay intended for the quantitative determination of enoxaparin in purified solutions by measurement of factor IIa 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 IIa 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-IIa activity is a necessary tool for monitoring treatment efficacy.
Presence of Enoxaparin catalyzes the reaction between AT lll and α-Thrombin. The factor IIa inhibition test is the most useful assay covering the widest variety of enoxaparin preparations. In the assay, the rate of factor IIa inhibition is directly proportional to the enoxaparin concentration since both factor IIa and AT-III are in excess. The residual factor IIa activity is inversely proportional to the enoxaparin concentration.

Materials Provided:

1. Human Anti-thrombin III Reagent(Lyophilized) – 2 vial
2. Human Thrombin-α Reagent(Lyophilized) - 1 vial
3. Chromogenic Substrate(Lyophilized) - 2 vial
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
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. **Human Thrombin-α 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-FIIa) 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 1.25 ml of Distilled water and leave it to stand for 15 minutes.
2. **Human Thrombin Reagent:**
   Human Thrombin Reagent is a lyophilized preparation. For Reconstitution, add 6 ml of Distilled water and leave it to stand for 15 minutes.
3. **Chromogenic Substrate:**
   Chromogenic Substrate is a lyophilized substrate specific for Factor IIa 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: 0.040 IU/ml, 0.030 IU/ml, 0.020 IU/ml and 0.010IU/ml.

For Example:

Preparation of Standard Concentrations

Standard Concentration 1400 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	100	35.71ul of M.S	464.29	500
S2	1	10ul of S1	990	1000
S3	0.040	40 ul of S2	960	1000
S4	0.030	30 ul of S2	970	1000
S5	0.020	20 ul of S2	980	1000
S6	0.010	10 ul of S2	990	1000

Test Dilution – Test Sample Main Stock is of concentration 1400 IU/mL

Sr No.	Concentration (IU/ml)	Stock (µL)	Diluent Buffer pH 7.4 (µL)	Total Volume (µL)
T1	100	35.71ul of M.S	464.29	500
T2	1	10ul of T1	990	1000
T3	0.040	40 ul of T2	960	1000
T4	0.030	30 ul of T2	970	1000
T5	0.020	20 ul of T2	980	1000
T6	0.010	10 ul of T2	990	1000

Assay Protocol:

Add the reagents into the microwell as per following steps:

	microwell
Standard or Test Sample	20µl
Anti-thrombin III	20µl
Mix but do not allow bubbles to form. Incubate at 37ºC, for 1 minute	
Human Thrombin-α	60µl
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 IIa 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 IIa 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.

LIMITED WARRANTY

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