KRISHZYME® Heparin Factor Xa Assay Kit

REF: KBBA04S

(100 tests)

Ver 5.7

RUO

Chromogenic assay for testing Heparins (UFH) in purified systems by measurement of Factor Xa inhibition, in compliance with EP Pharmacopoeia

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

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REF KBBA04S Σ 100 tests

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Intended Use:

Heparin Factor Xa is a chromogenic assay intended for the quantitative determination of unfractionated heparin (UFH) 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 heparin. This inhibition accounts for the anticoagulant effect of heparin. The quantitative determination of heparin levels by the measurement of their Anti-Xa activity is a necessary tool for monitoring treatment efficacy.

Unfractionated heparin (UFH) catalyzes both reactions equally. The Factor Xa inhibition test is the most useful assay covering the widest variety of heparin preparations. In the assay, the rate of Factor Xa inhibition is directly proportional to the heparin concentration since both Factor Xa and AT-III are in excess. The residual Factor Xa activity is inversely proportional to the heparin concentration.

Materials Provided:

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

Materials to be provided by the End-User:

1. Microplate Reader / Spectrophotometer able to measure absorbance at 405 nm.
2. Adjustable pipettes to measure volumes ranging from 25 ul to 2500 ul, duly calibrated.
3. Deionized (DI) water.
4. Parallel line software for data analysis.
5. Plastic tubes or cuvettes or microtiter plates with overflow capacity ≤ 350 ul/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:

1. **Human Anti-thrombin III Reagent:** Reconstituted reagent is stable for 2 weeks at 2-8°C and for 4 months at -20°C.

2. **Bovine Factor Xa Reagent:** Reconstituted reagent is stable for 2 weeks at 2-8°C and for 4 months at -20°C.

3. **Chromogenic Substrate:** Reconstituted reagent is stable for 2 weeks at 2-8°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 Heparin (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 heparin 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 2.5 ml of Factor Dilution buffer pH 8.4 and leave it to stand for 15 minutes. 15 minutes before running the experiment, prepare working solution by adding 500 ul of reconstituted Human Anti-thrombin III reagent with 500 ul of Factor Dilution buffer pH 8.4. Use this working solution for running assay.

2. **Bovine Factor Xa Reagent:**
Factor Xa Reagent is a lyophilized preparation. For Reconstitution, 2.5 ml of Factor Dilution buffer pH 8.4 and leave it to stand for 15 minutes. 15 minutes before running the experiment, prepare working solution by adding 160 ul of reconstituted Bovine Factor Xa Reagent with 840 ul of Factor Dilution buffer pH 8.4. Use this working solution for running assay.

3. **Chromogenic Substrate:**
Chromogenic Substrate is a lyophilized substrate specific for Factor Xa activity. For Reconstitution, add 2.5 ml of Distilled water and leave it to stand for 15 minutes. 15 minutes before running the experiment, prepare working solution by adding 250 ul of reconstituted Chromogenic Substrate with 750 ul of Distilled water. Use this working solution for running assay.

4. **Factor Dilution Buffer: For Standard / Sample and Reagents (Not provided in the kit) :**
Dissolve 6.10 g of tris (hydroxymethyl) aminomethane,10.20 g of sodium chloride, 2.80 g of EDTA sodium, and, if suitable, between 1% of macrogol 6000 and/or 2.00 g of bovine serum albumin in 800 ml of water. [NOTE - 2.00 g of human albumin may be substituted for 2.00 g of bovine serum albumin]. Adjust with hydrochloric acid to a pH of 8.4, and dilute with water to 1000 ml.

5. **20% (v/v) Acetic Acid Solution (Stop Solution):** (Not provided in the kit)
Prepare 20 % (v/v) Glacial Acetic Acid in Distilled Water to be used as a stop solution.

6. **Standard and Test Concentration:** Recommended range of standard and test
0.30 IU/ml, 0.225 IU/ml, 0.15 IU/ml, and 0.075 IU/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 (ul)	Diluent (Buffer pH 8.4) (ul)	Total Volume (ul)
S1	50	50 ul of M.S.	450	500
S2	1	20 ul of S1	980	1000
S3	0.30	90 ul of S2	210	300
S4	0.225	67.5 ul of S2	232.5	300
S5	0.15	45 ul of S2	255	300
S6	0.075	22.5 ul of S2	277.5	300

Test Dilution - Test Sample Main Stock is of concentration 500 IU/ml

Sr No.	Concentration (IU/ml)	Stock (ul)	Diluent (Buffer pH 8.4) (ul)	Total Volume (ul)
T1	50	50 ul of M.S.	450	500
T2	1	20 ul of T1	980	1000
T3	0.30	90 ul of T2	210	300
T4	0.225	67.5 ul of T2	232.5	300
T5	0.15	45 ul of T2	255	300
T6	0.075	22.5 ul of T2	277.5	300

Assay Protocol:

Add the reagents into the microwell as per following steps:

	Microwell
Human Anti-thrombin III	50 ul
Standard/Test Sample	50 ul
Mix but do not allow bubbles to form. Incubate at 37^{0}C, for 1 minute	
Bovine Factor Xa	100 ul
Mix and incubate at 37^{0}C, for exactly 2 minute	
Chromogenic Substrate	100 ul
Mix and incubate at 37^{0}C, for 4 minutes	
Stop Solution	50 ul
Mix and measure the absorbance at 405 nm	

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 heparins 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.

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