

KRISHZYME™ Heparin Factor IIa Assay Kit






REF: KBBA03S

(100 tests)

Ver5.1

RUO

Chromogenic assay for testing Heparins (UFH) in purified systems by measurement of Factor IIa 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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Intended Use:

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

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

Materials Provided:

1. Human Anti-Thrombin III Reagent - 4 vials (lyophilized)
2. Human α -Thrombin Reagent - 1 vial (lyophilized)
3. Chromogenic Substrate - 1 vial (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 μ l to 2500 μ l, duly calibrated
3. Deionized (DI) water
4. Parallel line software for data analysis
5. Plastic tubes or cuvettes or microtiter plates with overflow capacity \leq 350 μ l/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 α -Thrombin 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 Heparin (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 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 2.5ml of Distilled water and leave it to stand for 15 minutes.

2. Human α -Thrombin Reagent:

Human α -Thrombin Reagent is a lyophilized preparation. For Reconstitution, add 2.5ml 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 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 edetate 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

0.03 IU/ml, 0.02 IU/ml, 0.01 IU/ml, and 0.005 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 (μl)	Diluent (Buffer pH 8.4) (μl)	Total Volume (μl)
S1	50	50 μl of M.S	450	500
S2	1	20 μl of S1	980	1000
S3	0.03	18 μl of S2	582	600
S4	0.02	200 μl of S3	100	300
S5	0.01	100 μl of S3	200	300
S6	0.005	150 μl of S5	150	300

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

Sr No.	Concentration (IU/ml)	Stock (μl)	Diluent (Buffer pH 8.4) (μl)	Total Volume (μl)
T1	50	50 μl of M.S	450	500
T2	1	20 μl of T1	980	1000
T3	0.03	18 μl of T2	582	600
T4	0.02	200 μl of T3	100	300
T5	0.01	100 μl of T3	200	300
T6	0.005	150 μl of T5	150	300

Assay Protocol:

Add the reagents into the microwell as per following steps:

	Microwell
Human Anti-thrombin III	100 μl
Standard/Test Sample	50 μl
Mix but do not allow bubbles to form. Incubate at 37°C, for 1 minute	
Human α-Thrombin	25 μl
Mix and incubate at 37°C, for exactly 1 minute	
Chromogenic Substrate	50 μl
Mix and incubate at 37°C, for 4 minutes	
Acetic Acid	50 μl
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 heparins 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.

LIMITED WARRANTY

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