KRISHGEN BioSystems

Validation of KRISHZYME™ Factor IIa Assay for testing of Tinzaparin Sodium and Tinzaparin Injection

Introduction

KRISHZYME FIIa Assay kit is a chromogenic assay intended for the quantitative determination of Tinzaparin / Tinzaparin Sodium in purified solutions by measurement of factor IIa inhibition activity. The kit can be used for 100 test reactions as per microtiter plate protocol.

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 Tinzaparin. This inhibition accountsfor the anticoagulant effect of Tinzaparin.

The quantitative determination of Tinzaparin levels by the measurement of their anti-IIa activity is a necessary tool for monitoring treatment efficacy.

Presence of Tinzaparin catalyzes the reaction between factor IIa and AT-III. The factor IIa inhibition test is the most useful assay covering the widest variety of Tinzaparin preparations.

In the assay, the rate of factor IIa inhibition is directly proportional to the Tinzaparin concentration since both factor IIa and AT-III are in excess. The residual factor IIa activity is inversely proportional to the Tinzaparin concentration.

Materials and Method:

Materials provided in lyophilized form are :

Materials	Amount of DI water for reconstitution (ml)	After Reconstitution 1:4 dilution
Human Anti-thrombin III Reagent	1ml	125µl of M.S + 375µl of buffer
Human Thrombin-α Reagent	1ml	125µl of M.S + 375µl of buffer
Chromogenic Substrate	1ml	125µl of M.S + 375µl of H_2O

Note : M.S denotes Reconstituted Main stock from Tinzaparin Sodium EPRS

Materials not provided are :

Reagent	Materials Required
Dilution Buffer	20mM Tris, pH 7.4 and 150mM NaCl
Stop Solution	20% v/v Glacial Acetic Acid
Recommended Standard Concentration (considering 1mg=100IU)	0.48 IU/ml, 0.36 IU/ml, 0.24 IU/ml, and 0.12 IU/ml.

Assay Procedure :

Standard or Test Sample	50µl
Human Anti-thrombin III	50µl
Mix but do not allow bubbles to form. Incubate at 37ºC, for 2 minutes	
Human Thrombin-α	50µl
Mix and incubate at 37ºC, for exactly 2 minutes	
Chromogenic Substrate	50µl
Mix and incubate at 37ºC, for 2 minutes	
Acetic Acid	50µl
Mix and measure the absorbance at 405nm	

Standard and Test Sample Preparation : *Example* - Standard Concentration 100 IU/mL (Main Stock) is to be diluted as per below table:

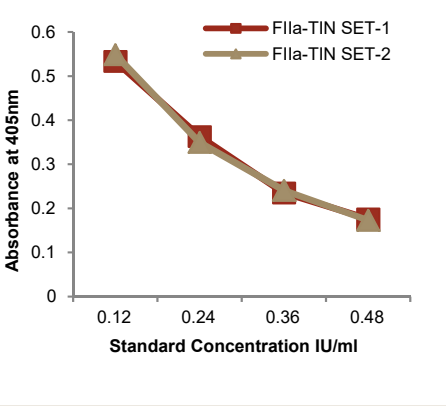
Sr.no	Concentration (IU/ml)	Stock(µl)	Diluent (µl) (buffer pH7.4)	Total Volume (µl)
S1	10	50 µl of Main Stock	450	500
S2	1	50 µl of S1	450	500
S3	0.48	240 µl of S2	260	500
S4	0.36	180 µl of S2	320	500
S5	0.24	120 µl of S2	380	500
S6	0.12	60 µl of S2	440	500

Test Dilution – Test Sample Main Stock is of concentration 100IU/ml

Sr.no	Concentration (IU/ml)	Stock (µl)	Diluent (µl) (buffer pH7.4)	Total Volume (µl)
T1	10	50 µl of Main Stock	450	500
T2	1	50 µl of T1	450	500
T3	0.48	240 µl of T2	260	500
T4	0.36	180 µl of T2	320	500
T5	0.24	120 µl of T2	380	500
T6	0.12	60 µl of T2	440	500

Results :

Standard Concentration IU/ml	Absorbance at 405nm	
	Set-1	Set-2
0.12	0.5338	0.5488
0.24	0.3633	0.3497
0.36	0.2352	0.2409
0.48	0.1764	0.1732



Data Interpretation :

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 Tinzaparin 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 softwarefor the same based on DJ Finney algorithm.

Conclusion :

The assay kits manufactured by KRISHGEN BIOSYSTEMS are validated Chromogenic Assays for the determination of Tinzaparin using anti-IIa activity in human plasma successfully met all standard assay-validation parameters and were suitablefor use inbioequivalencestudies.

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