KRIBIOLISA Tinzaparin anti F-Xa Assay kit, Cat # KBBA04T

KRISHGEN BioSystems

Validation of a chromogenic Factor Xa Assay for testing of Tinzaparin Sodium and Tinzaparin Injection

Introduction

Tinzaparin anti-FXa is a chromogenic assay intended for the quantitative determination of Tinzaparin in purified solutions by measurement of factor Xa 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 Xa and other coagulation serine proteases in plasma is increased several thousand-fold by Tinzaparin. This inhibition accounts for the anticoagulant effect of Tinzaparin.

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

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

In the assay, the rate of factor Xa inhibition is directly proportional to the Tinzaparin concentration since both factor Xa and AT-III are in excess. The residual factor Xa 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	100µl of M.S + 400µl of buffer
Bovine Factor Xa Reagent	1ml	100µl of M.S + 400µl of buffer
Chromogenic Substrate	1ml	100µl of M.S + 400µl of H_2O

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

Materials not provided are:

Reagent	Materials require
Dilution Buffer	20mM Tris, pH 7.4 and 150mM NaCl
Stop Solution	20% v/v Glacial Acetic Acid
Recommended Standard concentration (considering 1mg=100IU)	0.20 IU/ml, 0.15 IU/ml, 0.10 IU/ml, and 0.05 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	
Bovine Factor Xa	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:

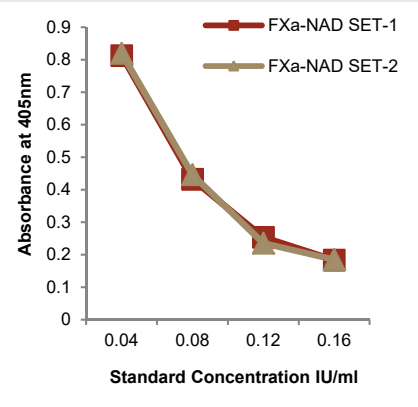
Sr.no	Concentration (IU/ml)	stock(µl)	Diluent (µl) (buffer pH7.4)	Total volume (µl)
S1	10	50 µl of Main Stock	450	500
S2	1	50 µl of S1	450	500
S3	0.20	100 µl of S2	400	500
S4	0.15	75 µl of S2	425	500
S5	0.10	50 µl of S2	450	500
S6	0.05	25 µl of S2	475	500

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	0.20	100 µl of T2	400	500
T4	0.15	75 µl of T2	425	500
T5	0.10	50 µl of T2	450	500
T6	0.05	25 µl of T2	475	500

Result :

Standard concentration IU/ml	Absorbance at 405nm	
	Set-1	Set-2
0.05	0.6875	0.6983
0.10	0.2886	0.42
0.15	0.2541	0.2396
0.20	0.1921	0.1604



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 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.

Conclusion :

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

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