KRISHZYME® Factor Xa Inhibitor Screening Assay Kit

REF : KBBA73

Ver1.0

RUO

Chromogenic Assay for Estimation of Factor Xa Inhibitor Activity in Human Blood Products

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 KBBA73 Σ 96 tests

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Introduction:

Factor X, also known as EC3.4.21.6 or thrombokinase, is a serine endopeptidase involved in blood coagulation. Factor X needs to be activated into Factor Xa to cleave prothrombin into active thrombin. Deficiency in inactive and active Factor X results in blood coagulation disorders. Warfarin, a common anticoagulant used in the treatment of thrombosis, inhibits the conversion of Factor X to Xa, resulting in lower risk of stroke and thrombosis.
Warfarin is used to treat pulmonary embolism and lower the risk of cardiovascular complications. The development of new Factor Xa inhibitors, also known as blood thinners, has been crucial in improving the quality of life of patients by bypassing the need for frequent blood tests.

Intended Use:

The KRISHZYME® Factor Xa Inhibitor Screening Assay Kit colorimetric assay designed to measure the activity of human Factor Xa for screening and profiling applications.

Principle:

The assay is based on a chromogenic method in which Factor Xa cleaves a specific synthetic substrate to release p-nitroaniline (pNA), producing a yellow colour measurable at 405 nm. To evaluate inhibitory effects, Factor Xa is preincubated with or without a test inhibitor prior to substrate addition. In the absence of an inhibitor, active Factor Xa cleaves the substrate efficiently, resulting in increased colour intensity, whereas in the presence of an inhibitor, enzymatic activity is reduced, leading to decreased pNA release and lower absorbance. Thus, the intensity of the colour is directly proportional to Factor Xa activity and inversely proportional to the inhibitory effect of the test compound.

Materials Provided:

1. Human Factor Xa (>1 ug) - 1 vial
2. Substrate – 50 ul
3. Dilution Buffer – 10 ml
4. 96 well plate - 1 Nos.
5. 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 10 ul to 2500 ul, duly calibrated.
3. 1.5 ml Eppendorf tubes.
4. Deionized (DI) water.
5. Software for data analysis, if required.
6. Timer/Stop watch.

Reagent Preparation, Storage, and Stability Information:

Human Factor Xa and the Substrate need to be stored in -80ºC and store the Dilution Buffer at -20ºC.

Note:

- Bring all reagents to room temperature.
- All reagents should be diluted immediately prior to use.

Preparation of test inhibitors:

a. If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations in Dilution Buffer.

b. If the Test inhibitor is soluble in DMSO, prepare the inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the inhibitor 10-fold in Dilution Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%. Use 10% DMSO in Dilution Buffer (vol/vol) as diluent for the serial dilution to keep the concentration of DMSO constant. For positive and negative controls, prepare 10% DMSO in Dilution Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

Health Hazard Warnings:

1. The source materials have 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. However adequate care should be taken when handling these materials as a source for potentially infective agents.
2. The Buffer contains Sodium Azide as preservative. It may be harmful if ingested, inhaled or absorbed through the skin. Refer to product MSDS for details.
3. For Research Purpose and In-Vitro Laboratory Use Only.

Reagent Preparation:

1. **Working Factor Xa Preparation:**
   a. Thaw Factor Xa on ice. Briefly spin the tube to recover the full content
   b. Dilute Factor Xa to 0.125 ng/ul in Dilution Buffer (40 ul/well).

Note: Keep the diluted protein on ice until use. Discard any unused diluted protein after use.

2. **Working Substrate Solution:**
   Dilute Substrate 100-fold in Dilution Buffer.

Assay Protocol:

Component	Negative Control	Positive Control	Test Inhibitor
Dilution Buffer	40 ul	-	-
Working Factor Xa	-	40 ul	40 ul
Test Inhibitor	-	-	10 ul
Dilution Buffer	10 ul	10 ul	-
30 minutes at Room Temperature			
Working Substrate	50 ul	50 ul	50 ul
Total	100 ul	100 ul	100 ul

1. After adding the Substrate, incubate the plate for 30 – 60 mins at room temperature OR perform Kinetic Analysis.
2. Take the reading at 405 nm.

Safety Precautions:

- **This kit is For Research Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents.
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.

- Since the kit contains potentially hazardous materials, the following precautions should be observed.

- Do not smoke, eat or drink while handling kit material.
- Always use protective gloves.
- Never pipette material by mouth.
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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