CREATIVE BIOMART®
Assay Kit

Factor IXa Activity Fluorometric Assay Kit

Product Information

Cat.No. Kit-0335

Product Overview

Factor IXa Activity Assay kit is based on the ability of FIXa to generate FXa. The generated FXa proteolytically cleaves a synthetic substrate and releases a fluorophore, AMC, which can be easily quantified by fluorescence microplate reader. The assay is simple, rapid and can detect activity as low as 10 pg of FIXa in a variety of samples.

Description

The coagulation Factor IX (or Christmas factor) (EC 3.4.21.22) is a vitamin K-dependent serine protease. Factor IX is produced as an inactive precursor and is activated via cleavage by either factor XIa (contact pathway) or factor VIIa (tissue factor pathway). In the presence of calcium ions and negatively charged membrane phospholipids, activated factor IX (FIXa) then binds to the activated Factor VIII (FVIIIa) and proteolytically activates factor X (FX) to factor Xa (FXa).

Applications

- Determine activity of pure FIXa
- Detect activity of FIXa in plasma, and serum

Storage

Store kit at -20°C, protected from light.

Kit Components

FIXa Assay Buffer 15 ml
FXa Substrate-AMC 0.2 ml
Enzyme Mix I 1 vial
Enzyme Mix II 1 vial
Phospholipids 0.6 ml
FIXa Enzyme Standard (10 ng) 1 vial

Materials Required but Not Supplied

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- 96-well white microplate with flat bottom
- Multi-well spectrophotometer.

Compatible Sample Types

- Purified enzyme
- Serum, plasma

Preparation

- FIXa Assay Buffer: Bring to room temperature before use.
- Enzyme Mix I: Reconstitute in 220 µl FIXa Assay Buffer. Mix well by pipetting up and down. Briefly centrifuge, aliquot & store at -20°C. Avoid repeated freeze/thaw.
- Enzyme Mix II: Reconstitute in 220 µl FIXa Assay Buffer. Mix well by pipetting up and down. Briefly centrifuge, aliquot & store at -80°C. Avoid repeated freeze/thaw.
- Phospholipid Vesicles: Vortex for 10 sec. before each use. Phospholipids can be stored at 4°C for one month. For long term storage -20°C is recommended. Avoid repeated freeze/thaw.
- FIXa Enzyme Standard: Reconstitute in 10 µl FIXa Assay Buffer to prepare a stock solution of 1 ng/µl. Mix well by pipetting up and down. Aliquot and store at -80°C. Avoid repeated freeze/thaw.

Assay Protocol

1. Sample Preparation: Dilute serum and plasma samples 10 times with FIXa Assay Buffer and add 2-10 µl/well into a 96-well plate in two wells (Sample Well (S) and Background Control Well (Bck)). For purified enzyme, add 2-10 µl (in the expected range of 10 to 500 pg) per well into desired well(s). Adjust the volume of background control and sample wells to 10µl/well with FIXa Assay Buffer.
Notes: a. For unknown samples, we suggest doing pilot experiment and testing several amounts of FIXa to ensure the readings are within the Standard Curve range.
b. Background control well is necessary to subtract basal Factor Xa activity that might be present in the sample.
2. Standard Curve: Prepare FIXa Enzyme Working Solution (10 pg/µl) just before use by adding 198 µl of FIXa Assay Buffer to 2 µl of FIXa Enzyme stock solution (1 ng/µl). Add 0, 2, 4, 6, 8, and 10 µl of FIXa Enzyme working solution (10 pg/µl) into a series of wells in a 96-well plate to prepare 0, 20, 40, 60, 80, and 100 pg/well of FIXa Enzyme Standard. Adjust the volume in all standard wells to 10µl with FIXa Assay Buffer.

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Note: Always make fresh FIXa Enzyme working solution.
3. Reaction Mix: Prepare a master mix of 10 µl/well by adding the components in the order shown:
Enzyme Mix I 2 µl
Phospholipids 6 µl
Enzyme Mix II 2 µl
Mix and add 10 µl of the master mix into each standard and sample well. Add 10 µl of FIXa Assay Buffer to all background control well(s). Adjust the volume to 98 µl/well with FIXa Assay Buffer. Mix well by pipetting up and down. Incubate for 15 min. at 37°C. After incubation, add 2 µl of FXa substrate-AMC into Standard, background control and sample wells. Mix well.
4. Measurement: Measure fluorescence in kinetic mode for 30-60 min. at 37°C (Ex/Em = 360/450 nm). Choose two time points (T1 and T2) in the linear range of the plot and obtain the corresponding values for the fluorescence (RFUs1 and RFUs2 and RFUbck1 and RFUbck2).
Note: Incubation time depends on the FIXa activity in the samples. We recommend measuring fluorescence in kinetic mode, and choosing two time points (T1 and T2) in the linear range to calculate the FIXa activity of the samples.

Analysis

5. Calculations: Subtract 0 Standard reading from all readings. Subtract the background signal from the sample signal. Plot the Factor IXa Standard Curve. Apply corrected sample's ΔRFU to Factor IXa Standard Curve to obtain corresponding Factor IXa (B, in ng) and calculate the activity of Factor IXa in the sample as:
Sample Factor IXa Activity = B/ V × Dilution Factor = pg / ml = ng/ L
Where, B is FIXa amount in the sample well from Standard Curve (pg)
V is sample volume added into the reaction well (ml)

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