Description

The Factor Xa Inhibitor Screening Assay is a colorimetric assay designed to measure the activity of human Factor Xa for screening and profiling applications. The assay kit comes in a convenient 384-well format that contains enough purified human Factor Xa, a chromogenic substrate, and PR-02 buffer for 400 reactions.

To determine the effect of an inhibitor on Factor Xa activity, the enzyme should be preincubated with or without the test inhibitor prior to adding the chromogenic substrate to the reaction. The assay was functionally validated using Rivaroxaban, a potent inhibitor of Factor Xa.

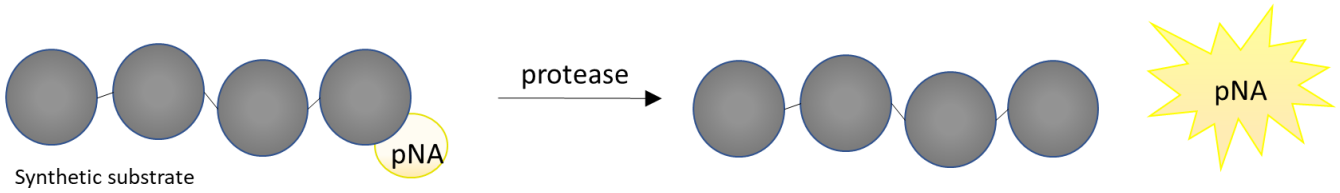

Figure 1: Illustration of the assay principle.
Upon proteolysis, Factor Xa cleaves the chromogenic substrate at the C-terminal end releasing p-nitroanilid (pNA), which produces a yellow color that is measurable photometrically at λ=405 nm. The increase in color is proportional to Factor Xa activity.

Background

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.

Applications

Screen small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
	Human Factor Xa*	> 1 µg	-80°C
	PR Substrate 2	2 x 50 µl	-80°C
	PR-02 Buffer	2 x 10 ml	-20°C
	96-well transparent microplate	1	Room Temp

** The concentration of protein is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- UV/Vis microplate reader capable of reading λ=405 nm
- Rotating or rocker platform

Stability

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include a "Negative Control", "Positive Control" and "Test inhibitor."
- If the assay plate is going to be used more than once, prepare enough reagents for this portion of the assay and aliquot the remaining undiluted reagents into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C or at -20°C as appropriate.

1. Thaw **Human Factor Xa** on ice. Briefly spin the tube to recover the full content.

2. Dilute Factor Xa to 0.125 ng/µl in **PR-02 Buffer** (20 µl/well).

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

3. Prepare the Test Inhibitor (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   a) If the Test Inhibitor is water-soluble, prepare serial dilutions 10-fold more concentrated than the desired final concentrations in PR-02 Buffer. PR-02 Buffer is the Diluent Solution.

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 100-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in PR-02 Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   Use 10% DMSO in PR-02 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 PR-02 Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO should not exceed 1%.

4. Add 20 µl of diluted Factor Xa to all wells except to "Negative Control".

5. Add 20 µl of PR-02 Buffer to the "Negative Control" wells.

6. Add 5 µl of inhibitor solution to the "Test Inhibitor" wells.

7. Add 5 µl of Diluent Solution to the "Positive Control" and the "Negative Control" wells.

8. Preincubate the "Test inhibitor" with the diluted Factor Xa for 30 minutes at Room Temperature (RT) with gentle agitation.

9. Dilute **PR Substrate 2** 100-fold in PR-02 buffer.

10. Add 25 µl of the diluted Substrate to all wells.

Component	**Negative control**	**Positive Control**	**Test Inhibitor**
PR-02 Buffer	20 µl	-	-
Diluted Factor Xa (0.125 ng/µl)	-	20 µl	20 µl
Test inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
30 minutes at Room Temperature			
Diluted PR Substrate 2	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

11. Incubate at RT for 30-60 minutes or perform kinetic analysis.

12. Read the plate at λ=405 nm in an appropriate microplate reader.

Example Results

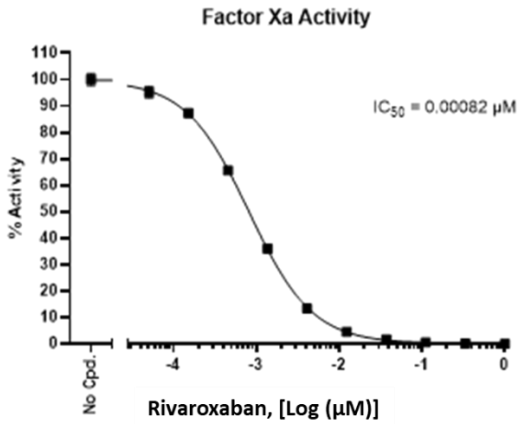

Figure 1. Human Factor Xa activity is inhibited by Rivaroxaban.
Factor Xa activity was measured in the presence of increasing concentrations of Rivaroxaban (MedChemExpress # HY-50903). Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor and set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Thrombin Inhibitor Screening Assay Kit	78867	96 reactions/384 reactions