Description

The Thrombin Inhibitor Screening Assay is a colorimetric assay designed to measure the activity of human alpha thrombin for screening and profiling applications. The assay kit comes in a convenient 96-well format and contains enough purified human alpha thrombin, a chromogenic substrate, and PR-02 buffer for 100 reactions.

To determine the effect of an inhibitor on Thrombin 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 Dabigatran, a potent inhibitor of thrombin.

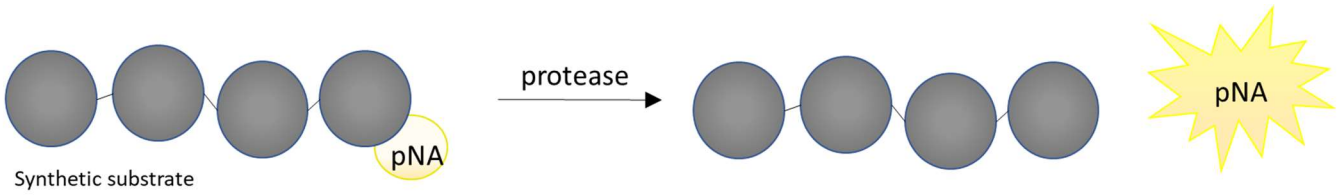

Figure 1: Illustration of the assay principle.
Upon proteolysis, thrombin cleaves the chromogenic substrate at the C-terminal end releasing p-nitroanilid (pNA), which produces a yellow color that is measurable photometrically at λ=405 nm. The increase in color is proportional to thrombin activity.

Background

Thrombin (also known as factor IIa, activated blood-coagulation factor II, EC 3.4.21.5 or fibrogenase) is a serine protease crucial for blood coagulation. Thrombin is synthetized in the inactive form prothrombin, and it is cleaved by activated Factor X (Xa). It activates factor XI, VIII, V XIII, and converts fibrinogen to fibrin by cleaving fibrinogen chains into monomers. Activated factor XIII (XIIIa) increases the stability of the clot by forming covalent bonds between fibrin molecules. In addition, thrombin leads to platelet activation and aggregation. Interestingly, thrombin is also involved in a negative feedback mechanism, by acting as an inhibitor of the coagulation cascade when bound to thrombomodulin. Thrombin is involved in the formation of blood clots in arteries, which can result in cerebral ischemia and stroke. It is also involved in atherosclerosis via angiogenesis (vascular cell recruitment to the plaque), and inflammation. The use of small molecule inhibitors for thrombin is a viable therapy in cardiovascular complications.

Applications

Screen small molecule inhibitors that bind directly to the catalytic site of thrombin in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
82887	Human alpha thrombin*	5 µg	-80°C
82888	PR Substrate 2	50 µl	-80°C
82773	PR-02 Buffer	10 ml	-20°C
79963	Transparent 96-well 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".
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Dabigatran as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Thaw **human alpha thrombin** on ice. Briefly spin the tube to recover the full content.

2. Dilute Thrombin to 1.25 ng/µl in **PR-02 Buffer** (40 µl/well).

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

3. Prepare the Test Inhibitor (10 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 100 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 inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired concentration, 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 40 µl of diluted Thrombin to all wells except "Negative Control".
5. Add 40 µl of PR-2 Buffer to the "Negative Control" wells.
6. Add 10 µl of inhibitor solution to the "Test Inhibitor" wells.
7. Add 10 µl of Diluent Solution to the "Positive Control" and "Negative Control" wells.
8. Preincubate the "Test inhibitor" with the diluted thrombin for 30 minutes at Room Temperature (RT) with gentle agitation.
9. Dilute **PR Substrate 2** 100-fold in PR-02 Buffer (50 µl/well).
10. Initiate the reaction by adding 50 µl of the diluted PR Substrate 2 to all wells.
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.

Component	**Negative Control**	**Positive Control**	**Test Inhibitor**
PR-02 Buffer	40 µl	-	-
Diluted Thrombin (1.25 ng/µl)	-	40 µl	40 µl
Test inhibitor	-	-	10 µl
Diluent Solution	10 µl	10 µl	-
30 minutes at Room Temperature			
Diluted PR Substrate 2	50 µl	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

Example Results

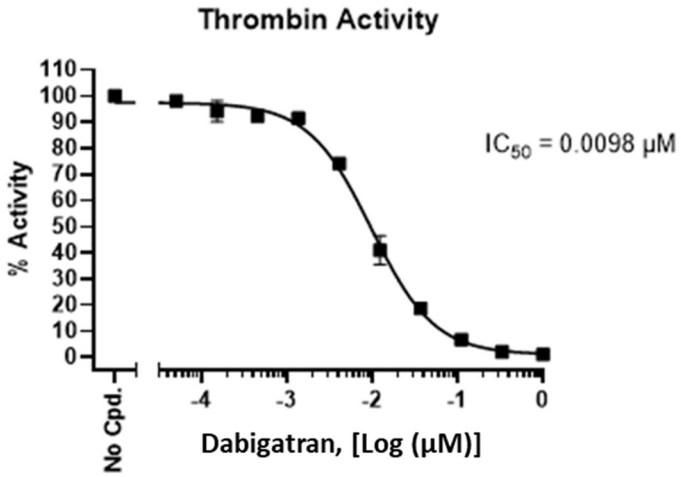

Figure 1. Human alpha thrombin activity is inhibited by Dabigatran.
Thrombin activity was measured in the presence of increasing concentrations of Dabigatran (MedChemExpress #HY-10163). Results are expressed as percentage of activity relative to the positive control (measured in the absence of inhibitor 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*
Factor Xa Inhibitor Screening Assay Kit	78868	96 reactions/384 reactions

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