Description

The Activated Protein C (APC, also known as PROC) Assay Kit is a 96-well format fluorogenic assay designed to measure the protease activity of Activated Protein C (PROC) for screening and profiling applications. The kit contains sufficient amounts of purified recombinant PROC protein, PROC fluorogenic substrate, and PROC assay buffer for 100 enzyme reactions.

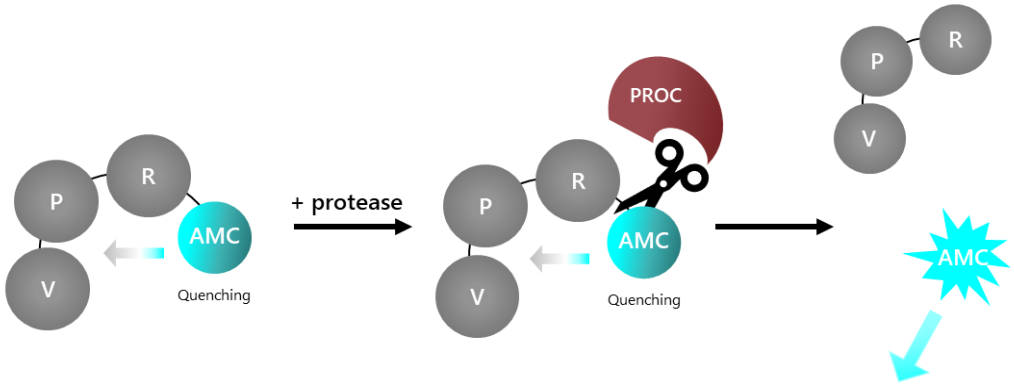

Illustration of the assay principle.

The substrate is an internally quenched fluorogenic substrate. Proteolysis releases the highly fluorescent AMC. Fluorescence intensity increases proportionally to the activity of the protease.

Background

Protein C, also known as autoprothrombin IIA and blood coagulation factor XIX, is a serine protease which acts as an anticoagulant and is normally expressed in its zymogen (pro-enzyme) form. Activated protein C (APC or PROC) inhibits coagulation by inactivating the proteolysis of Factor Va and Factor VIIIa and regulating blood vessel permeability and inflammatory responses. PROC activation is vitamin-K dependent and vitamin K deficiency or alterations in PROC activation can lead to increased risk of blood clot-related pathologies such as thrombosis.

Applications

Study enzyme kinetics and screen small molecule inhibitors for drug discovery and high throughput applications.

Supplied Materials

Catalog #	Name	Amount	Storage
70019	Activated Protein C (PROC), His-Tag*	13 µg	-80°C
	PROC Assay Buffer	5 ml	-20°C
	PROC Fluorogenic Substrate (20 mM)	25 µl	-80°C
79685	Black 96-well plate	1	Room Temperature

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

Materials Required but Not Supplied

Name	Catalog #
Fluorescence plate reader capable of reading *exc* = 380 nm; *em* = 460 nm	
Adjustable micropipettor and sterile tips	
30°C incubator	

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.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

All samples and controls should be tested in duplicate.

1. Thaw **PROC Assay Buffer.**

2. 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.

   If the Test Inhibitor is water-soluble:

   2.1 Prepare serial dilutions in the **PROC Assay Buffer**, 10-fold more concentrated than the desired final concentrations.

   2.2 For the positive and negative controls, use **PROC Assay Buffer** (Diluent Solution).

   Or

   If the Test inhibitor is soluble in DMSO:

   2.1 Prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 10-fold in **PROC Assay Buffer** to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

   2.2 Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in **PROC Assay Buffer** to keep the concentration of DMSO constant.

   2.3 For positive and negative controls, prepare 10% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

3. Add 5 µl of **Test Inhibitor** to each well labeled “Test Inhibitor.” For the “Positive Control" and “Blank,” add 5 µl of **Diluent Solution** (either PROC Assay Buffer or 10% DMSO in PROC Assay Buffer, as described above).

4. To the wells designated as "Blank," add 20 µl of **PROC Assay Buffer**.

5. Thaw **Activated Protein C (PROC)** on ice. Briefly spin the tube to recover its full contents. Dilute the protein to 6.25 ng/µl using **PROC Assay Buffer** (you need 20 µl/well).

   Note: The concentration of protein is lot-specific and is indicated on the tube. Verify the initial concentration and dilute accordingly. Avoid multiple freeze/thaw cycles. Do not re-use the diluted protein.

8. Add 20 µl of diluted **Activated Protein C (PROC)** to the wells designated "Positive Control" and "Test Inhibitor."

9. Thaw **PROC Fluorogenic Substrate (20 mM).** Prepare the **PROC Fluorogenic Substrate (20 mM)** by diluting 1:100 in **PROC Assay Buffer**, creating **PROC Fluorogenic Substrate (200 µM)**.

Protect the fluorogenic peptide and the reaction from direct exposure to light.

10. Initiate the reaction by adding 25 µl of **PROC Fluorogenic Substrate (200 µM)** to the wells labeled "Blank," "Positive," and "Test Inhibitor."

Component	Blank	Positive Control	Test Inhibitor
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
PROC Assay Buffer	20 µl	-	-
Activated Protein C (PROC); 6.25 ng/µl	-	20 µl	20 µl
PROC Fluorogenic Substrate (200 µM)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

11. Incubate at room temperature for 30 minutes.

12. Measure the fluorescence intensity in a microtiter plate-reading fluorimeter capable of excitation at a wavelength of 380 nm and detection of emission at a wavelength of 460 nm. The fluorescence intensity can also be measured kinetically. "Blank" value is subtracted from all other values.

Example Results

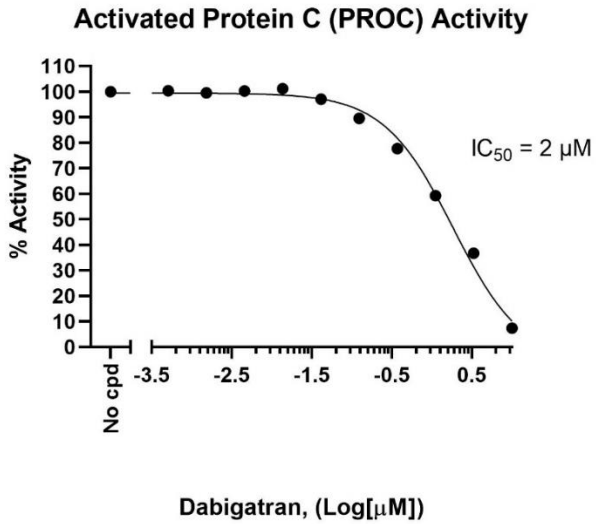

Figure 1: Inhibition of Activated Protein C (PROC) Activity by Dabigatran (MedChemExpress #HY-10163).
The activity of Activated Protein C (PROC) was measured in the presence of increasing inhibitor concentrations. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (protease activity in the absence of inhibitor, set at 100%).

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

Troubleshooting Guide
Visit Assay-kits-faq for detailed troubleshooting instructions. For further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
Activated Protein C (PROC), His-Tag Recombinant	70019	20 µg