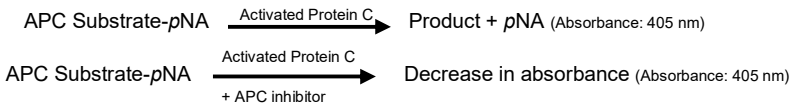
Activated Protein C (APC) Inhibitor Screening Kit (Colorimetric) (#BN00672)

(Catalog # BN00672; 100 assays; Store at -20°C)

I. Introduction:

Activated protein C (APC) (3.4.21.69), a Vitamin K dependent serine protease, is formed by activation of Protein C by Thrombomodulin bound on the surface of endothelial cells. By degrading coagulation factors Va and VIIIa, APC inhibits blood coagulation which helps in the prevention of thrombosis. Blood coagulation can be stimulated by inhibition of APC. Thus, APC inhibitors play an important role in the treatment of disease like Haemophilia. Assay Genie's APC Inhibitor Screening Kit can be used to screen for potent inhibitors of APC activity. It utilizes the proteolytic activity of an active APC on a peptide substrate which releases a colored product. The released product can be easily quantified using an absorbance microplate reader. In the presence of an APC inhibitor, the enzyme decreases/loses activity resulting in lower/no absorbance. This assay kit is simple and can be used to identify and characterize APC inhibitors in a high-throughput format. As a control, we provide the inhibitor PPACK dihydrochloride - a known APC inhibitor.



II. Applications:

- Screening/characterizing inhibitors/ligands of APC

III. Kit Contents:

Components	BN00672	Cap Code	Part Number
APC Assay Buffer	25 ml	WM	BN00672-1
APC Enzyme	1 vial	Green	BN00672-2
APC Substrate	400 µl	Brown	BN00672-3
APC Inhibitor (1 mM PPACK Dihydrochloride)	50 µl	Blue	BN00672-4

IV. User Supplied Reagents and Equipment:

- 96-well clear plate with flat bottom
- Multi-well Absorbance microplate reader

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **APC Assay Buffer:** Store at -20 °C or 4 °C. Bring to room temperature before use.
- **APC Enzyme:** Store at -20°C. Reconstitute by adding 500 µl APC Assay buffer per tube before use and aliquot. Once reconstituted, use within two months. Avoid multiple freeze thaws.
- **APC Substrate:** Ready to use. Store at -20°C. Thaw and aliquot before use. Avoid multiple freeze thaw cycles.
- **APC Inhibitor:** Ready to use. Store at -20°C. Thaw before use. Avoid multiple freeze/thaw of the inhibitor.

VI. APC Inhibitor Screening Protocol:

1. **APC Enzyme Working Solution Preparation**: To each well (Enzyme Control-EC, Sample-S, Inhibitor Control-IC and Solvent Control-SC, Background Control -BC), add:

	BC	EC	S, SC and IC
APC Assay Buffer	45 µl	45 µl	40 µl
APC Enzyme	---	5 µl	5 µl

Prepare enough reagents. Mix well.

2. **Screening Compounds, Inhibitor Control & Enzyme Control Preparations:** Dissolve candidate inhibitors at 20X highest final test concentration using preferred solvent. Add 5 µl of test inhibitors (S, BC), APC Inhibitor (IC) or inhibitor solvent (SC) to respective wells and incubate at Room temperature (RT) for 10 min.

   Note: High solvent concentration might affect the enzymatic activity. Prepare parallel well(s) as Solvent Control to test the effect of the solvent on enzyme activity (same as EC in presence of final solvent concentration).

3. **APC Substrate:** Prepare enough reagents for the number of assays to be performed. For each well, prepare 50 µl of the Substrate Mix:

   46 µl APC Assay Buffer
   4 µl APC Substrate

   Mix & add 50 µl of APC Substrate Mix into each BC, EC, S, SC and IC wells. Mix well.

4. **Measurement:** Measure absorbance at 405 nm in a kinetic mode for 1 hr at room temperature.

5. **Calculations**: Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the Absorbance (Ab_1 and Ab_2). Calculate the slope for all samples, $\Delta Ab/\Delta t$ after subtracting the Background Control (BC) change for the same Δt (in case SC values are significantly different from EC values use the SC values in the equations below):.

$$\%\ \textbf{Relative activity} = \frac{\textbf{Δ Ab/Δt of S}}{\textbf{Δ Ab/Δt of EC}} \times \textbf{100}$$

$$\%\ \textbf{Relative inhibition} = \frac{\textbf{AAB/}\Delta\textbf{t of EC} - \textbf{AAb/}\Delta\textbf{t of S}}{\Delta\ \textbf{Ab/}\Delta\textbf{t of EC}} \times 100$$

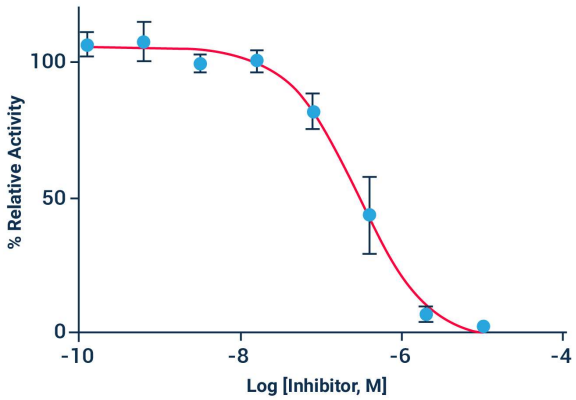


Figure: Inhibition of APC activity by APC Inhibitor, IC_{50} = 286 nM (n = 3). Assay was performed following the kit protocol.

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