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Product Information

Activated Protein C (APC) Inhibitor Screening Kit

Catalog Number **MAK346**
Storage Temperature –20 °C

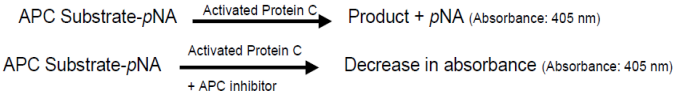
TECHNICAL BULLETIN

Product Description

Activated protein C (APC), 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 hemophilia.

The Activated Proctein C (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 by measuring absorbance at 405 nm using a 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, the inhibitor PPACK dihydrochloride is provided, a known APC inhibitor.



Components

The kit is sufficient for 100 colorimetric assays in 96 well plates.

APC Assay Buffer Catalog Number MAK346A	25 mL
APC Enzyme Catalog Number MAK346B	1 vial
APC Substrate Catalog Number MAK346C	400 µL
APC Inhibitor (1 mM PPACK Dihydrochloride) Catalog Number MAK346D	50 µL

Reagents and Equipment Required but Not Provided.

- Pipetting devices and accessories (e.g., multichannel pipettor)
- 96 well clear plate with flat bottom
- Spectrophotometric multiwell plate reader

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at –20 °C, protected from light upon receiving. Briefly centrifuge small vials prior to opening.

Preparation Instructions.

Reagent Preparation

APC Assay Buffer – Bring to room temperature before use.

APC Enzyme – Reconstitute by adding 500 µL of APC Assay buffer per tube before use and aliquot. Once reconstituted, use within two months. Avoid multiple freeze thaws.

APC Substrate – Thaw and aliquot before use. Avoid multiple freeze/thaw cycles.

APC Inhibitor – Thaw before use. Avoid multiple freeze/ thaw cycles.

Procedure

APC Inhibitor Screening

APC Enzyme Working Solution – Reactions:

Enzyme Control (EC)
Sample (S)
Inhibitor Control (IC)
Solvent Control (SC)
Background Control (BC)

Prepare the appropriate APC Enzyme Working Solution (see Table 1). Prepare volume required for the number of reactions. Mix well. Add indicated volume (see Table 1) to appropriate well.

Table 1.
Preparation of APC Enzyme Working Solution

Reagent	BC	EC	S, SC, and IC
APC Assay Buffer	45 µL	45 µL	40 µL
APC Enzyme	–	5 µL	5 µL

Screening Compounds, Inhibitor Control and Enzyme Control Preparations – Dissolve candidate inhibitors at 20× 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 for 10 minutes.

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).

APC Substrate Mix – Prepare enough reagent for the number of assays to be performed. For each well, prepare 50 µL of the APC Substrate Mix:

46 µL APC Assay Buffer
4 µL APC Substrate

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

Measurement – Measure absorbance (A_{405} nm) in kinetic mode for 1 hour at room temperature.

Results

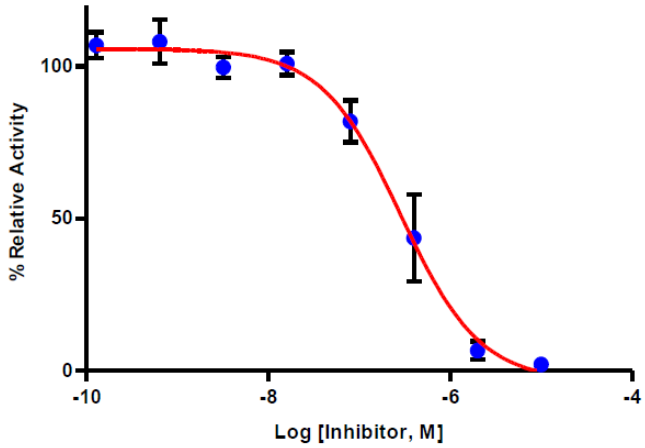
1. Choose two time points (t_1 & t_2) in the linear range of the plot and obtain the corresponding values for the absorbance (A_1 and A_2).
2. Calculate the slope for all samples, $\Delta A/\Delta t$ after subtracting the Background Control (BC) change for the same Δt:

$$\% \text{ Relative Activity} = \frac{\Delta A/\Delta t \text{ of S}}{\Delta A/\Delta t \text{ of EC}} \times 100$$

$$\% \text{ Relative Inhibition} = \frac{\Delta A/\Delta t \text{ of EC} - \Delta A/\Delta t \text{ of S}}{\Delta A/\Delta t \text{ of EC}} \times 100$$

Note: In case SC values are significantly different from EC values, use the SC values in the equations.

Figure 1.
APC Activity Inhibition



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

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