3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Forskolin

Catalog Number **F3917**
Store at Room Temperature

CAS RN 66575-29-9

Product Description
Molecular Formula: $C_{22}H_{34}O_7$
Molecular Weight: 410.50
Source: *Coleus forskohlii*

T cell activation is normally triggered by the interaction of a cell surface receptor to its specific ligand molecule. This binding event triggers the rapid hydrolysis of inositol phospholipids to diacylglycerol and inositol phosphates by phospholipase C (PLC). Diacylglycerol, an allosteric activator of protein kinase C (PKC) and inositol phosphates, which trigger Ca^{2+} release and mobilization, result in a cascade of additional cellular responses mediating T cell activation. One of these cellular responses is the production and secretion of interleukin-2 (IL-2). Phorbol 12-myristate 13-acetate (PMA), which has a structure analogous to diacylglycerol, can also activate PKC.

Jurkat cells are a leukemic T cell line known to produce IL-2. Under normal growth conditions, little to no IL-2 is produced in Jurkat cells. PMA, through its activation of PKC, can activate T cells and stimulate a low-level of IL-2 production. When Jurkat cells are stimulated by PMA and a co-stimulator, such as phytohemagglutinin (PHA), IL-2 production is strongly enhanced.[1] PHA by itself can trigger a low level of T cell activation and IL-2 production by binding non-specifically to the cell surface receptor complex, although the combination of PMA and PHA results in greatly increased IL-2 production. In T lymphocytes, the activation of adenylate cyclase by forskolin is known to inhibit the synthesis of IL-2.[2]

Forskolin appears to interfere with this process by indirectly interfering with the activation of phospholipase C.[3] Forskolin activates adenylate cyclase, the enzyme that converts adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP). cAMP is an activator of protein kinase A (PKA). PKA, however, has been shown to phosphorylate a serine residue on PLC, which is thought to indirectly cause its inactivation.

Phospholipase C is normally activated by phosphorylation at multiple tyrosine residues. Phosphorylation of the serine residue by PKA seems to interfere with activation of PLC by tyrosine phosphorylation.

Precautions and Disclaimer
This product is 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.

Preparation Instructions
Forskolin is soluble in organic solvents such as ethanol, chloroform, and DMSO. Sigma routinely tests its solubility in chloroform at 50 mg/mL and observes a clear, colorless to pale yellow solution.

Forskolin is soluble in water (with 2% ethanol) up to 0.2 mM by first dissolving in ethanol at 5 mg/mL and doing subsequent dilutions with water. Similar results were found with DMSO. Please note, however, that various solvents, including ethanol, inhibited the forskolin activation of adenylate cyclase. DMSO is recommended for preparing forskolin solutions because at concentrations of 5% or less of DMSO, there is little if any inhibition of forskolin activation.

Storage/Stability
Store the product at room temperature. All stock solutions should be stored in the dark at –20 °C.

Procedure

Assay

2.5 mL of Jurkat cells (1×10^6 cells/mL) and 2.5 mL fresh culture medium (RPMI-1640 with 10% fetal calf serum containing 10 mL/L penicillin-streptomycin) were added to 25 cm^2 culture bottles. The following additions were made in duplicate.

a. **Control** - no additions
b. **1 µg/mL PHA + 10 ng/mL PMA**
   Add 10 µL of PHA stock solution (0.5 mg/mL PHA in filter-sterilized PBS) and 5 µL of PMA stock solution (10 µg/mL PMA in DMSO)
c. **0.2 mM Forskolin + 1 µg/mL PHA + 10 ng/mL PMA**
   Add 10 µL of PHA stock solution, 5 µL of PMA stock solution, and 10 µL of forskolin stock solution (100 mM forskolin in DMSO)

After mixing well, the bottles were incubated at 37 °C for 24 hours. After centrifugation, the clarified broth was then tested for IL-2 production by ELISA assay. IL-2 production in the test cultures containing 0.2 mM forskolin was inhibited ≥50% compared to the test cultures containing only PHA and PMA.

Results

In the presence of 1 µg/mL PHA and 10 ng/mL PMA plus 0.2 mM forskolin, production of IL-2 was inhibited ≥50% as compared to control cells containing no forskolin.

References

1. Manger, B., *et al*., J. Clin. Invest., **77**, 1501 (1986).
2. Minakuchi, R., *et al*., J. Immunol., **145**, 2616 (1990).
3. Park, D.J., *et al*., J. Biol. Chem., **267**, 1496 (1992).

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