

## Product Information

### FORSKOLIN

Product Number **F 3917**  
Storage at Room Temperature

CAS #: 66575-29-9

#### Product Description

Molecular Formula:  $C_{22}H_{34}O_7$   
Molecular Weight: 410.5  
Purity: Minimum 98%  
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, 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.<sup>1</sup> 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.<sup>2</sup>

Forskolin appears to interfere with this process by indirectly interfering with the activation of phospholipase C.<sup>3</sup> 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 phosphoryl-

ation at multiple tyrosine residues. Phosphorylation of the serine residue by PKA seems to interfere with activation of PLC by tyrosine phosphorylation.

#### Reagent

Forskolin is supplied as a powder.

#### Preparation Instructions

Soluble in DMSO (see suitability assay below). For other applications, forskolin may also be solubilized in ethanol.

#### Storage/Stability

All stock solutions should be stored in the dark at  $-20\text{ }^{\circ}\text{C}$

#### Procedure

##### Suitability Assay

2.5 ml of Jurkat cells ( $1 \times 10^6$  cells/ml) and 2.5 ml fresh culture media (RPMI-1640 + 10% fetal calf serum containing 10 ml/l penicillin-streptomycin) were added to 25  $\text{cm}^2$  culture bottles. The following additions were made in duplicate.

- Control** - no additions
- 1  $\mu\text{g/ml}$  PHA + 10  $\text{ng/ml}$  PMA**  
Add 10  $\mu\text{l}$  PHA stock solution (0.5 mg/ml PHA in filter-sterilized PBS) + 5  $\mu\text{l}$  PMA stock solution (10  $\mu\text{g/ml}$  PMA in DMSO)
- 0.2 mM Forskolin + 1  $\mu\text{g/ml}$  PHA + 10  $\text{ng/ml}$  PMA**  
Add 10  $\mu\text{l}$  PHA stock solution + 5  $\mu\text{l}$  PMA stock solution + 10  $\mu\text{l}$  forskolin stock solution (100 mM forskolin in DMSO)

After mixing well, the bottles were incubated at  $37\text{ }^{\circ}\text{C}$  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  $\geq 50\%$  compared to the test cultures containing only PHA and PMA.

**Product Profile**

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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