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Product Information

Cyclosporin A from *Tolypocladium inflatum*
for molecular biology

Catalog Number **C1832**
Storage Temperature 2-8 °C

CAS RN: 59865-13-3

Synonyms: Cyclosporine; Antibiotic S 7481F1; Ciclosporin; CsA

Molecular formula: $C_{62}H_{111}N_{11}O_{12}$
Molecular weight: 1202.61

Melting point: 148-151 °C [1]
$[\alpha]^{20}_{D}$: –244° (c = 0.6 in chloroform)[1]

Product Description
Cyclosporin A is a non-polar cyclic oligopeptide produced by the fungus *Tolypocladium inflatum*. It is a potent immunosuppressive agent, affecting primarily T-lymphocytes.[2] It has been shown to inhibit the functioning of several nuclear proteins involved in T-cell activation at the level of mRNA transcription.[3] It forms a complex with its intracellular receptor cyclophilin, which can then bind to calcineurin, a Ca^{2+}- and calmodulin-dependent protein phosphatase, inhibiting its enzymatic activity.[4,5,6] CsA was found to suppress the replication of hepatitis C virus genome in cultured hepatocytes.[7] At concentrations >10 nM, CsA protected isolated hepatocytes against the action of phalloidin.[8] CsA can inhibit IL_2 production resulting from T cell activation via Calcineurin inhibition.

An extensive list of references has been reported, including a comprehensive review of analytical properties.[9]

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sigma solubility assays involve only organic solvents: 10 mg/mL in methylene chloride, 6 mg/mL in chloroform, 10 mg/mL in ethanol, 50 mg/mL in DMSO. Solutions are clear, colorless to faint yellow. It is also soluble in methanol and acetone. Cyclosporin is slightly soluble in water and saturated hydrocarbons.[1]

Storage/Stability
Store dessicated and protected from light at 2-8 °C. Under these conditions the product is stable for 2 years. It should be re-evaluated for suitability in user's application every two years. Stock solutions in ethanol or DMSO should be stored at –20 °C. Cyclosporin is stable in solution if protected from light, but its concentration may drop due to adsorption to the container walls.

Product Summary
In the presence of 1 μg/ml PHA, 50 ng/ml PMA and 1 μg/ml Cyclosporin A, production of IL-2 was inhibited at least 90% compared to control cells containing no Cyclosporin A.

Suitability Assay
2.5 ml of Jurkat cell culture (1 x 10^6 cells/ml) and 2.5 ml fresh culture medium (RPMI 1640 + 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 + 50 ng/ml PMA
   Add 10 μL PHA stock solution
   (0.5 mg/ml PHA in filter sterilized PBS)
   and 2.5 μL PMA stock solution
   (100 μg/ml PMA in DMSO)
c. 1 μg/ml Cyclosporin A + 1 μg/ml PHA + 50 ng/ml PMA
   Add 5 μL Cyclosporin A stock solution
   (1 mg/ml Cyclosporin A in ethanol) + 10 μL PHA stock solution + 2.5 μL PMA stock solution

After mixing well the bottles were incubated at 37 °C for 24 hours. After centrifugation, the clarified broth was tested for IL-2 production using an ELISA assay. IL-2 production in the test cultures containing 1 μg/ml Cyclosporin A was inhibited at least 90% compared to the test cultures containing only PHA and PMA.

References
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3. Emmel, E.A., et al., Science, **246,** 1617-20 (1989)
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