Forskolin

Cat. No.: HY-15371
CAS No.: 66575-29-9
Molecular Formula: C₂₂H₃₄O₇
Molecular Weight: 410.5
Target: Adenylate Cyclase; Autophagy
Pathway: GPCR/G Protein; Autophagy
Storage:
- Powder
  - -20°C: 3 years
  - 4°C: 2 years
- In solvent
  - -80°C: 6 months
  - -20°C: 1 month

**SOLVENT & SOLUBILITY**

**In Vitro**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Mass Concentration</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>100 mg/mL (243.61 mM; Need ultrasonic)</td>
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<tr>
<td>H₂O</td>
<td>&lt; 0.1 mg/mL (insoluble)</td>
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</tbody>
</table>

Preparing Stock Solutions

- 1 mM: 2.4361 mL
- 5 mM: 0.4872 mL
- 10 mM: 0.2436 mL

Please refer to the solubility information to select the appropriate solvent.

**In Vivo**

1. Add each solvent one by one: 10% DMSO >> 40% PEG300 >> 5% Tween-80 >> 45% saline
   - Solubility: ≥ 2.5 mg/mL (6.09 mM); Clear solution
2. Add each solvent one by one: 10% DMSO >> 90% corn oil
   - Solubility: ≥ 2.5 mg/mL (6.09 mM); Clear solution

**BIOLOGICAL ACTIVITY**

Description

Forskolin is a potent adenylate cyclase activator, with IC₅₀ and EC₅₀ of 41 nM and 0.5 μM for type I adenylyl cyclase, respectively.

IC₅₀ & Target

IC₅₀: 41 nM (Adenylyl cyclase)[1]
EC₅₀: 0.5 μM (Adenylyl cyclase)[1]

In Vitro

Forskolin (Fsk) is a naturally occurring diterpene isolated from Coleus forskohlii, directly activates adenylyl cyclase (AC) through its catalytic subunit to increase intracellular levels of cyclic adenosine monophosphate (cAMP)[1].
Forskolin (Fsk) affects the proliferation of the human T-cell lines such as Kit 225 and MT-2. Forskolin treatment inhibits the proliferation of both Kit 225 and MT-2 cells in a dose-dependent manner with an IC\textsubscript{50} equal to ~5 µM Fsk. Forskolin treatment (10-100 µM) increases cAMPi levels ~5- to 20-fold above basal levels, which reach maximum levels between 50-100 µM Forskolin\cite{2}.

**In Vivo**

The Forskolin (Fsk)-treated Mrp4\textsuperscript{-/-} mice shows an increased number of Ki67-positive and cleaved caspase 3-positive ECs, a significant decrease in the amount of pericyte coverage, and a reduced number of empty sleeves. In pups exposed to hyperoxia (75% oxygen) from P7 to P12, the Mrp4\textsuperscript{-/-} mice shows a significant increase in the unvascularized retinal area\cite{3}. The average blood glucose in the healthy rat group is 102.12±1.94 mg/dL, 101.25±3.56 for control group and 103±2.08 in forskolin group. The data shows that glucose levels at the end of the study are lower in forskolin group, with a significant difference according to the statistical tests applied (p=0.03)\cite{4}.

**PROTOCOL**

**Kinase Assay**\cite{2}

For Jak3 kinase assays, Fsk-treated MT-2 cells are lysed, clarified, and immunoprecipitated using Jak3 antibody. Kinase reactions are carried out at 30°C for 20 min. For PKA kinase assays, untreated MT-2 cells are lysed, and Jak3 is immunoprecipitated and bound to PAS beads. Immunoprecipitated Jak3 is washed with kinase buffer (50 mM Hepes-NaOH (pH 7.4), 10 mM MgCl\textsubscript{2}, 0.5 mM EGTA, 0.5 mM DTT, 20 µg/mL aprotinin, 10 µg/mL leupeptin, 1 µg/mL pepstatin A) and incubated with 200 µM ATP and purified protein kinase A catalytic subunit (PKAc) as indicated in the figure legends. Kinase reactions are carried out at 32 °C for 30 min followed by vigorous washing of the beads with cold kinase wash buffer. For [\textsuperscript{γ-32P}]ATP radiolabeled kinase assays using recombinant Jak3, Hek293 cells are transfected with wild type (WT) Jak3 or kinase-dead Jak3 K855A using Lipofectamine 2000 according to the manufacturer's instructions. Cells are lysed and immunoprecipitated with Jak3 antibody. Jak3-bound PAS beads are washed three times in cold lysis buffer followed by kinase reactions. Kinase reactions are initiated by adding 10 µCi [\textsuperscript{γ-32P}]ATP, 10 µM unlabeled ATP, and 1 µg of purified PKAc to Jak3-bound PAS bead reaction mixtures. Kinase reactions are performed at 32°C for 30 min. Jak3-bound PAS beads are washed three times in radioimmunoaassay buffer (10 mM Tris-HCl, pH 7.4, 75 mM NaCl, 20 mM EDTA, 10 mM EGTA, 20 mM Na\textsubscript{4}P\textsubscript{2}O\textsubscript{7}, 50 mM NaF, 20 mM 2-glycerolphosphate, 1 mM p-nitrophenolphosphate, 0.1% Triton X-100) and one time in kinase wash buffer. The reactions are stopped by adding 2× SDS-PAGE sample buffer followed by SDS-PAGE. Coomassie stainable Jak3 bands are excised from the PVDF membrane and subjected to phosphoamino acid analysis\cite{2}.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**Cell Assay**\cite{2}

Quiescent Kit 225 or MT-2 cells are seeded into 96-well plates at 5×10\textsuperscript{4} cells per well. Cells are then pretreated for 1 h with 1% DMSO (vehicle) or Forskolin at 1, 5, 10, 25, 50, and 100 µM concentrations. The cells are stimulated with IL-2 and cultured for an additional 20 h at 37°C. Control cells are treated with 1% DMSO for 20 h. During the final 4 h of incubation, the cells are pulsed with \textsuperscript{[3H]}thymidine at a concentration of 0.5 µCi/200 µL. Cells are harvested onto fiberglass filters and analyzed using liquid scintillation counting\cite{2}.

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**Animal Administration**\cite{3,4}

C57BL/6J mice are used. Mrp4-knockout mice, which are established and repeatedly backcrossed to the C57BL/6J mice. Forskolin is injected intraperitoneally into neonatal mice at postnatal days 4 (P4) and 5 (P5). Mice injected with DMSO serve as the controls. The treated mice are euthanized at P6, and their retinas are isolated for whole-mount immunohistochemistry (IHC). The effect of different concentrations of Forskolin on the survival rate and retinal vasculature is first tested, and the optimal concentration is determined, 1.0 µg/50 µL (0.3 mg/kg) at P4 and 1.5 µg/50 µL (0.5 mg/kg) at P5, used to compare the retinal vascular phenotypes between WT mice and Mrp4-deficient mice.

Male Wistar rats, aged 10-14 weeks old, with a mean weight of 300 g±50 g, are divided into four groups; 19 are experimentally induced to develop diabetes, and 8 are maintained in a healthy condition. Both diabetic and healthy rats receive no Forskolin (control), or 6 mg/kg per day of Forskolin, administered orally for 8 weeks. Blood glucose
levels are determined in each group before and after Forskolin treatment. The diabetic rats are tested two weeks after confirming the presence of diabetes (three weeks after the induction) and after eight weeks of the designated treatment.

MCE has not independently confirmed the accuracy of these methods. They are for reference only.

**REFERENCES**


**CUSTOMER VALIDATION**

- Biomaterials. 2018 Dec 6;193:30-46.

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