Ampkinone

**Cat. No.:** HY-12831  
**CAS No.:** 1233082-79-5  
**Molecular Formula:** C₃₁H₂₃NO₆  
**Molecular Weight:** 505.52  
**Target:** AMPK  
**Pathway:** Epigenetics; PI3K/Akt/mTOR

**Storage:**  
- Powder: -20°C, 3 years; 4°C, 2 years  
- In solvent: -80°C, 6 months; -20°C, 1 month

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### SOLVENT & SOLUBILITY

#### In Vitro

DMSO: 50 mg/mL (98.91 mM; Need ultrasonic)

<table>
<thead>
<tr>
<th>Preparing Stock Solutions</th>
<th>Solvent Concentration</th>
<th>Mass</th>
<th>1 mg</th>
<th>5 mg</th>
<th>10 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mM</td>
<td></td>
<td>1.9782 mL</td>
<td>9.8908 mL</td>
<td>19.7816 mL</td>
</tr>
<tr>
<td></td>
<td>5 mM</td>
<td></td>
<td>0.3956 mL</td>
<td>1.9782 mL</td>
<td>3.9563 mL</td>
</tr>
<tr>
<td></td>
<td>10 mM</td>
<td></td>
<td>0.1978 mL</td>
<td>0.9891 mL</td>
<td>1.9782 mL</td>
</tr>
</tbody>
</table>

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 (4.95 mM); Suspended solution; Need ultrasonic

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### BIOLOGICAL ACTIVITY

**Description**  
Ampkinone is an indirect AMP-activated protein kinase (AMPK) activator.

**IC₅₀ & Target**  
AMPK

**In Vitro**  
Ampkinone stimulates the phosphorylation of AMPK via the indirect activation of AMPK in various cell lines. Ampkinone-mediated activation of AMPK requires the activity of LKB1 and results in increased glucose uptake in muscle cells[1].

**In Vivo**  
Ampkinone-treated DIO mice significantly reduce total body weight and overall fat mass. Histological examination and measurement of lipid parameters show that Ampkinone effectively improves metabolic abnormalities in the DIO mice model[1].
PROTOCOL

Kinase Assay

Total AMPK activity is measured using a synthetic SAMS peptide substrate and [γ-32P]ATP. Briefly, 500 μg of protein extract is incubated with anti-AMPK-α1 and α2 antibodies for 2 h at 4°C. Protein A/G sepharose and agarose are added, and the mixtures are incubated for 3 h at 4°C. After the samples are washed three times with RIPA buffer, the activity is assessed in AMPK reaction buffer containing 20 mM HEPES-NaOH (pH 7.0), 0.4 mM Dithiothreitol, and 0.01% Brij-35, with or without 300 μM AMP. The immune complexes are added to 23 μL of reaction buffer per assay, and then 7 μL of SAMS substrate peptide (HMRSAMGLHLVKRR, 100 μM final concentration) and 10 μL of ATP mixture (an aliquot of 1 μCi/μL: 90 μL of 75 mM magnesium chloride, 500 μM unlabeled ATP in 20 mM MOPS, pH 7.2, 25 mM β-glycerophosphate, 5 mM EGTA, 1 mM sodium orthovanadate, and 1 mM Dithiothreitol) are added, and the mixture is incubated at 30°C for 15 min. After 35 μL is spotted onto the center of P81 paper, the paper is washed three times with 0.75% phosphoric acid and once with acetone for 5 min. The samples are read using a scintillation counter.

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

Animal Administration

Mice

C57BL/6J mice are used and housed individually in a room maintained at 25°C on a 12/12 h light/dark schedule. For diet-induced obesity (DIO) mice, 4-week-old male C57BL/6J mice are fed a high-fat diet (HFD, 60% calories from fat) ad libitum for 8 weeks. Ampkinone in polyethylene glycol (PEG400) or vehicle is administered subcutaneously at 10 mg/kg body weight per day for 1 month. Body weight and food intake are measured every 3 days. All animals are given an insulin tolerance test (ITT) and sacrificed at 30 days. Liver weight and fat masses are measured, sectioned, and stained with Oil-red O and H&E.

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REFERENCES