Ac-YVAD-cmk
Caspase-1 inhibitor
Catalog # inh-yvad

For research use only
Version # 16E02-MM

PRODUCT INFORMATION
Contents:
• 5 mg Ac-YVAD-cmk
Storage and stability:
- Ac-YVAD-cmk is provided as a powder and shipped at room temperature. Store at -20°C. Ac-YVAD-cmk powder is stable for 2 years when properly stored.
- Upon resuspension, prepare aliquots of Ac-YVAD-cmk and store at -20°C. Resuspended Ac-YVAD-cmk is stable for 6 months when properly stored.
Quality control:
- Purity ≥97% (UHPLC)
- The inhibitory activity of the product has been validated using the in vitro inhibition of caspase-1 assay.
- The absence of bacterial contamination (e.g. lipopolysaccharide and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION
Ac-YVAD-cmk is a cell-permeable, selective and irreversible inhibitor of the cysteine protease caspase-1, also known as ICE (Interleukin-1-Converting Enzyme), with some activity against caspase-4 and caspase-5. Caspases-1, -4 and -5 belong to a family of inflammatory caspases (the group I caspases) that are crucial for regulating cell death and inflammation. Ac-YVAD-cmk is a chloromethyl ketone tetrapeptide based on the target sequence in pro-IL-1β (YVHD). As an inhibitor of caspase-1 activity, Ac-YVAD-cmk has been shown to effectively block apoptosis in a plethora of experimental paradigms, such as growth factor withdrawal, excitotoxicity, axotomy, cerebral ischemia and brain trauma. Furthermore, Ac-YVAD-cmk was reported to block inflammasome activation, and hence to display anti-inflammatory, anti-apoptotic and anti-pyroptotic effects.


CHEMICAL PROPERTIES
Solubility: 50 mg/ml (92.4 mM) in DMSO
Synonym: Ac-Tyr-Val-Ala-Asp-chloromethylketone
CAS number: 178603-78-6
Formula: C24H33ClN4O8
Molecular weight: 541
Structure:

METHODS
Preparation of 10 mg/ml (18.5 mM) stock solution
- Add 500 μl of DMSO to 5 mg Ac-YVAD-cmk. Mix by vortexing.
- Prepare further dilutions with endotoxin-free water.

Working concentration: 0.1–30 μg/ml for cell culture assays

In vitro inhibition of caspase-1:
The following protocol describes the monitoring of caspase-1 inhibition in human THP1-Null cells by assaying the inhibition of IL-1β production.
1. Pre-incubate THP-1 cells (3 x 10⁶ cells/well) with Ac-YVAD-cmk (0.1-30 μg/ml) in a 96-well plate for 1 hour at 37°C in 5% CO₂.
2. Prime cells by adding 1 μg/ml LPS for 3 hours at 37°C in 5% CO₂.
3. Wash cells gently with PBS and add fresh culture medium.
4. Stimulate cells by adding IL-1β inducers, such as ATP (5 mM) or MSU crystals (100-200 mg/ml), in the presence or absence of Ac-YVAD-cmk (0.1-30 μg/ml).
5. Incubate from 6 hours to overnight at 37°C in 5% CO₂.
6. Determine caspase-1 inhibition by detecting mature IL-1β in the supernatant of THP-1 cells by Western blot; or by ELISA, using a kit such as LumiKine™ hIL-1β; or with InvivoGen’s HEK-Blue™ IL-1β reporter cells, which are specifically engineered to detect bioactive IL-1β.

RELATED PRODUCTS

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<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Catalog Code</th>
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<tbody>
<tr>
<td>ATP</td>
<td>Adenosine 5’-triphosphate</td>
<td>ttrl-atp</td>
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<tr>
<td>HEK-Blue™ IL-1β cells</td>
<td>IL-1β reporter cells</td>
<td>hkb-il1b</td>
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<td>LumiKine™ hIL-1β</td>
<td>Bioluminescent ELISA kit</td>
<td>lumi-hi1b</td>
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<td>MSU Crystals</td>
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<td>VX-765</td>
<td>Caspase-1 inhibitor</td>
<td>ttrl-vx765-1</td>
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<tr>
<td>Z-VAD-FMK</td>
<td>Pan-caspase inhibitor</td>
<td>ttrl-vad</td>
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PROTOCOLS
For reference only; as described in the indicated publications.

Cell Culture Assay
Cells: Bone marrow cells
Working concentration: 50 μM (27 μg/ml)
Incubation time: 30 minutes
Method: Detection of pyroptosis by measuring released lactate dehydrogenase activity

Animal Study
Animal model: BALB/c mice
Dose: 8 mg/kg
Administration: Intraperitoneal injection