General Caspase Inhibitor
Q-VD-OPh

Catalog Number: OPH001

Reagent Specifications

Inhibitor Sequence: Q-Val-Asp(non-omethylated)-OPh
Molecular Weight: 513.77 Da
Quantity: 1 mg/vial
Activity: Measured by its ability to inhibit DEVD-AFC cleavage activity in cell extracts activated by the addition of cytochrome c and dATP. The IC_{50} for this effect is typically < 300 nM. See Activity Assay Protocol on the next page.

Storage

Store reagent desiccated at 2 - 8 °C upon arrival. Lyophilized, samples are stable for 1 year, desiccated, at 2 - 8 °C. Upon reconstitution in 100% DMSO, stock concentrations of 10 - 20 mM are stable for 6 months at ≤ -20 °C. Avoid repeated freeze/thaw cycles by aliquoting the stock solution prior to freezing.

Instructions for Use

Note: *Concentrations/Volumes listed below are based upon a single 1 mg vial. Adjust accordingly if using a different amount.*

Reconstitution: Inhibitor must be reconstituted using highly pure (ACS grade) DMSO. A pellet may not be visible at the bottom of the vial. Make sure the reagent is thoroughly dissolved in solution before use. Addition of 195 µL of DMSO to the vial will yield a 10 mM stock solution.

Cell Cultures: Cells may be cultured as required to induce apoptosis. Caspase inhibitors are typically added at the beginning of the cell culture process. Since inhibitors are dissolved in DMSO, a concentration of solvent above 1.0% may cause cellular toxicity thus masking the effect of the Caspase Inhibitor. For best results, working dilutions are prepared by direct dilution of a 10 mM stock solution into the culture medium prior to adding medium to cells. The following table may be used as a guide for dilutions. If the assay requires a greater concentration of inhibitor, increasing the stock concentration is recommended or alternatively, include a solvent control to monitor any DMSO-related effect(s). Q-VD-OPh is typically used in *in vitro* cell culture to inhibit apoptosis at final working concentrations of 10 - 100 µM. This variability is largely dependent on cell type, apoptotic signal and length of culture. The investigator must establish the most effective inhibitor concentration for their particular assay. Cells can then be harvested and tested for evidence of apoptosis using standard apoptosis assays.

Final Concentration of Inhibitor	Dilution into Cell Culture: 20 mM stock	10 mM stock
100 µM	**1:200**	**1:100**
20 µM	**1:1000**	**1:500**
10 µM	**1:2000**	**1:1000**

Animal Studies: For *in vivo* applications the recommended dose is 20 mg/kg. Q-VD-OPh is administered IP in 80 - 100% DMSO. Doses up to 120 mg/kg in mice have been reported without toxicity (4, 8).

Optimal dilutions should be determined by the laboratory for each specific applications.

Intended Use

Q-VD-OPh is a cell-permeable, irreversible inhibitor of caspase activity. The IC_{50} range for caspases -1, -3, -8, -9, -10, and -12 is 25 - 400 nM. Q-VD-OPh does not exhibit toxic effects at typical working concentrations: 10 - 20 µM for *in vitro* cell culture applications, 10 - 120 mg/kg for *in vivo* animal studies.

Principle of the Test

Cells that are induced to undergo apoptosis can be cultured in the presence or absence of Q-VD-OPh inhibitor. The Caspase Inhibitor binds to the active site of activated proteases. Treated cells can then be assayed for evidence of apoptosis inhibition by examining either whole cells or cell lysates using standard apoptosis assays.

Background Information

Members of the caspase gene family (cysteine proteases with aspartate specificity) play significant roles in both inflammation and apoptosis. Caspases exhibit catalytic and substrate-recognition motifs that have been highly conserved (1). These characteristic amino acid sequences allow caspases to interact with both positive and negative regulators of their activity (1). The substrate preferences or specificities of individual caspases have been exploited for the development of peptides that successfully compete for caspase binding (1 - 3). In addition to their distinctive aspartate cleavage sites at the P1 position, the catalytic domains of the caspases require at least four amino acids to the left of the cleavage site with P4 as the prominent specificity-determining residue (3). It is possible to generate reversible or irreversible inhibitors of caspase activation by coupling caspase-specific peptides to certain aldehyde, nitrile or ketone compounds. Q-VD-OPh (quinolyl-valyl-O-methylaspartyl-[-2, 6-difluorophenoxy]-methyl ketone) is a cell permeable, irreversible, broad-spectrum Caspase Inhibitor (4 - 8). Q-VD-OPh is more effective in preventing apoptosis than the widely used pan Caspase Inhibitor, ZVAD-fmk (5). Q-VD-OPh is effective in preventing apoptosis mediated by three major apoptotic pathways, caspase 9/3, caspase 8/10 and caspase 12 (5). Replacement of the fluoromethyl ketone (fmk) with a carboxy terminal O-Phenoxy group significantly reduced toxicity, even at high levels (5). The reduced toxicity and effective inhibition of caspases makes Q-VD-OPh a useful tool for apoptosis research using *in vitro* and *in vivo* models (4 - 8). Caspase inhibitors are important tools in the investigation of many biologic processes utilizing whole cells, cell lysates, and *in vivo* systems.

References

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R&D Systems, Inc.
1-800-343-7475

Activity Assay Protocol - Activation of Caspase Inhibitor in Cell Extracts of Jurkat A3 wild type cells

Materials

- Jurkat E6 wild type cell extracts (see supplementary methods for preparation)
- Extraction Buffer: 50 mM HEPES, 10 mM KCl, 5 mM EGTA, 1 mM $MgCl_2$, 0.2% CHAPS, 0.2 mM DTT, pH 7.5
- Assay Buffer: 10 mM HEPES, 0.5 mM EGTA, 5 mM DTT, 10% Glycerol, pH 7.5
- General Caspase Inhibitor Q-VD-Oph (R&D Systems, Catalog # OPH001)
- Cytochrome C, Bovine heart (Sigma, Catalog # C3131), 2 mg/mL stock in deionized water
- dATP (Sigma, Catalog # D6500), 10 mM stock adjusted to pH 7.5
- Substrate: Ac-Asp-Glu-Val-Asp-AFC (DEVD-AFC) (MP Biomedicals, Catalog # AFC138), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

Note: All reagents and assay components should be kept on ice until use.

1. Thaw cell extracts and centrifuge in a microcentrifuge at 14,000 rpms for 5 minutes at 4 °C. Transfer supernatants to chilled tubes and use within 1 hour.
2. Prepare a 10 mM stock of Q-VD-Oph in DMSO.
3. Prepare a curve of Q-VD-Oph (MW: 513.77 Da) in Extraction Buffer. Make the following serial dilutions: 7500, 2500, 750, 250, 75, 25, 7.5, 2.5, 0.75, and 0.25 nM.
4. Prepare the activator mixture by combining equal volumes of 2 mg/mL Cytochrome C and 10 mM dATP for final concentrations of 1 mg/mL and 5 mM, respectively.
5. Prepare reaction mixtures in tubes by combining 10 µL of each Q-VD-Oph curve dilution, 10 µL of cell extract supernatant, and 5 µL of the cytochrome C/dATP activator mixture. Also, prepare the following controls: #1) Total Control: 10 µL of Extraction Buffer, 10 µL of cell extract supernatant, and 5 µL of the cytochrome C/dATP activator mixture. #2) Inactive Control: 15 µL of Extraction Buffer and 10 µL of cell extract supernatant. The total reaction volume is 25 µL.
6. Incubate for 60 minutes at 30 °C.
7. After incubation, add 100 µL of Assay Buffer to each vial for a 1/5 dilution. Mix briefly.
8. Dilute Substrate to 100 µM in Assay Buffer.
9. In a plate load 50 µL of diluted incubated reaction mixtures, and start the reaction by adding 50 µL of 100 µM Substrate.
10. Read at excitation and emission wavelengths of 400 nm and 505 nm, respectively, in kinetic mode for 5 minutes.
11. Derive the 50% inhibiting concentration (IC_{50}) of Q-VD-Oph by plotting RFU/min vs. reaction concentration of Q-VD-Oph (step 5) with 4-PL fitting.

Final Assay Conditions Per Reaction

Q-VD-Oph curve: 3000, 1000, 300, 100, 30, 10, 3, 1, 0.3 and 0.1 nM
bCytochrome C: 0.2 mg/mL
dATP: 1 mM