**VX-765**
Caspase-1 inhibitor
Catalog code: inh-vx765i-1, inh-vx765i-5

https://www.invivogen.com/vx765

For research use only
Version 19C22-MM

**PRODUCT INFORMATION**

Contents
- VX-765 is available in two quantities:
  - 10 mg VX-765 (#inh-vx765i-1)
  - 5 x 10 mg VX-765 (#inh-vx765i-5)

Note: VX-765 is provided as a translucent film.

Storage and stability
- VX-765 is shipped at room temperature.
- Upon receipt, VX-765 should be stored at -20°C.
- Resuspended product is stable for 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control
- The inhibitory activity of the product has been validated using the in vitro inhibition of caspase-1 assay described overleaf.
- The absence of bacterial contamination (e.g. endotoxins and peptidoglycans) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

**CHEMICAL PROPERTIES**

CAS number: 273404-37-8

Chemical Name: (S)-1-((S)-2-[(1-(4-amino-3-chloro-phenyl)-methanoyl]-amino)-3,3-dimethyl-butanoyl)-pyrrolidine-2-carboxylic acid ((2R,3S)-2-ethoxy-5-oxo-tetrahydro-furan-3-yl)-amide

Solubility: 100 mg/ml (200 mM) in DMSO or ethanol

Formula: C24H33ClN4O6

Molecular weight: 509

Purity: >97% (HPLC)

Structure:

![Chemical Structure](image)

**DESCRIPTION**

VX-765 is an orally absorbed prodrug of VRT-043198, a potent and selective inhibitor of caspases belonging to the ICE/caspase-1 subfamily. VX-765 is converted to VRT-043198 under the action of plasma and liver esterases. The active metabolite of VX-765 exhibits potent inhibition of caspase-1 and caspase-4 and at least 100-fold lower potency against other non-ICE subfamily caspases. VX-765 was shown to reduce the production of IL-1β and IL-18 both in vitro and in vivo in correlation with tissue-protective effects in animal models of inflammatory disease. VX-765 was found in a phase IIa trial to be safe and well tolerated. Recent data demonstrate that VX-765 prevents CD4 T-cell death in a dose-dependent manner in HIV-infected lymphoid tissues.


**METHOD**

**Preparation of stock solution**
- Add the appropriate amount of DMSO to 10 mg of VX-765 and mix by vortexing:
  - for a 10 mg/ml stock solution, add 1 ml DMSO
  - for a 25 mM stock solution, add 786 µl DMSO
- Prepare further dilutions using H2O or PBS.

**Working concentration:** 0.1-50 µg/ml (200 nM-100 µM) for cell culture assays

**PROTOCOLS** (For reference only)

**Cell Culture Assay**
- Cells: CD4 T cells isolated from HLAC (human lymphoid aggregate culture)
- Working concentration: 10 µM
- Pre-incubation time: 4 hours
- Method: Fluorescent labelled inhibitors of caspases (FLICA) probes (cell-permeable probes that bind to active forms of specific caspases)

**Animal Study**
- Animal model: Naive male CD-1 mice
- Dose: 10, 21, 43, and 84 mg/kg
- Administration: Oral gavage
- Solubility: 25% Cremophor EL in water
**In vitro inhibition of caspase-1:**
The following protocol describes the monitoring of caspase-1 inhibition by VX-765 in the human monocytic THP1-Null cell line by measuring the inhibition of IL-1β production. The cells are grown in RPMI 1640 medium supplemented with 10% heat inactivated fetal bovine serum. THP1-Null cells are grown in suspension to a density of 1.0x10⁶ cells/ml in tissue culture flasks.

1. Pre-incubate THP-1 cells (3x10⁵ cells/well) with VX-765 (0.1-50 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 µg/ml), in the presence or absence of VX-765 (0.1-50 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, ELISA or with InvivoGen’s HEK-Blue™ IL-1β cells, which are specifically engineered to detect bioactive IL-1β.

**RELATED PRODUCTS**

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<tr>
<th>Product</th>
<th>Description</th>
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<td>ATP</td>
<td>Adenosine 5’-triphosphate</td>
<td>tlr-atp</td>
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<td>HEK-Blue™ IL-1β Cells</td>
<td>IL-1β reporter cells</td>
<td>hkb-il1b</td>
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<td>MSU Crystals</td>
<td>Monosodium urate crystals</td>
<td>tlr-msu</td>
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<td>THP1-Null Cells</td>
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<td>Z-VAD-FMK</td>
<td>Pan-caspase inhibitor</td>
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