Bafilomycin A1
Autophagy Inhibitor; V-ATPase inhibitor
Catalog code: tlrl-baf1
https://www.invivogen.com/bafilomycin-a1

For research use only
Version 21F21-MM

PRODUCT INFORMATION
Contents
• 10 µg of Bafilomycin A1 (BafA1)

Storage and stability
- Bafilomycin A1 is provided lyophilized and shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, store at -20°C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality Control:
- Inhibitory activity has been confirmed using cellular assays.
- The absence of bacterial contamination (e.g., lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION
Bafilomycin A1 (BafA1), a macrolide antibiotic isolated from the Streptomyces species, is a specific vacuolar H^+ ATPase (V-ATPase) inhibitor. BafA1 prevents the maturation of autophagic vacuoles by inhibiting late-stage fusion between autophagosomes and lysosomes as well as lysosomal degradation. Therefore, it is frequently used to study functional autophagy.

V-ATPases establish and maintain a low luminal pH in endocytic and exocytic compartments. Upon binding to the V-ATPase complex, BafA1 inhibits H^+ translocation, thereby depriving acidic intracellular compartments (i.e., endosomes, lysosomes, and vesicles) of H^+ ions, increasing their pH and inhibiting the function of resident hydrolases. Indeed, BafA1 inhibits the activation of nucleic acid sensing endosomal Toll-Like receptors (TLRs), such as TLR9, by neutralizing endosomal pH. On the other hand, this can lead to an accumulation of H^+ in the cytoplasm of treated cells, inducing acidosis and thus, can cause secondary adverse effects in normal cells. There is evidence demonstrating that BafA1 suppresses the growth of a variety of cancer cells by inhibiting autophagy and inducing apoptotic cell death via various mechanisms. An acidic pH is an important feature of the tumor microenvironment and a major determinant of tumor progression, and it is well-established that cancer cells upregulate autophagy as a survival mechanism. Therefore, inhibition of autophagy by BafA1, in combination with anti-cancer therapies, represents a promising therapeutic approach.


CHEMICAL PROPERTIES
CAS number: 88899-55-2
Formula: C_{35}H_{58}O_{9}
Molecular weight: 622.83 g/mol
Solubility: 0.1 mg/ml in DMSO or ethanol
Purity: ≥90% (UHPLC)
Structure:

METHODS
Preparation of 100 µM stock solution
2. Vortex until completely resuspended.
3. Prepare aliquots and store at -20°C. Once BafA1 has been resuspended, dilutions can be prepared with aqueous buffers.

PROTOCOL FOR V-ATPase INHIBITION IN HEK-BLUE™ hTLR9 CELLS
Below is a protocol for monitoring V-ATPase inhibition by BafA1 using InvivoGen’s HEK-Blue™ hTLR9 cells. These cells are specifically designed for the study of human Toll-like Receptor 9 (TLR9)-induced NF-κB signaling pathway by monitoring the activity of secreted embryonic alkaline phosphatase (SEAP) reporter activity. Changes in SEAP expression levels due to V-ATPase inhibition can be readily assessed using QUANTI-Blue™ Solution. For more information: https://www.invivogen.com/hek-blue-htrl9.

1. Add 20 µl of BafA1 (final concentration 100 nM to 1 µM) per well of a flat-bottom 96-well plate.
2. Add 160 µl of cell suspension (~80,000 cells) per well.
3. Add 20 µl of a test sample or a TLR9 agonist, such as ODN 2006 (final concentration 0.3 µg/ml) per well.
4. Incubate the plate for 18-24 hours at 37 °C in 5% CO2.
5. Determine inhibition by assessing SEAP expression using a SEAP detection medium, such as QUANTI-Blue™ Solution.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Description</th>
<th>Cat.Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEK-Blue™ hTLR9 Cells</td>
<td>Reporter cells</td>
<td>hkb-htrl9</td>
</tr>
<tr>
<td>ODN 2006 (ODN 7909)</td>
<td>Human TLR9 agonist</td>
<td>tlr-odn2006</td>
</tr>
<tr>
<td>Wortmannin</td>
<td>Autophagy inhibitor</td>
<td>tlr-wtm</td>
</tr>
<tr>
<td>QUANTI-Blue™ Solution</td>
<td>SEAP detection reagent</td>
<td>rep-qbs</td>
</tr>
</tbody>
</table>

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Asia: +852 3622-3480
E-mail: info@invivogen.com

https://www.invivogen.com