Bafilomycin A1

Autophagy Inhibitor; V-ATPase inhibitor - InvitroFit™

Catalog code: tlrl-baf1, tlrl-baf1-10

https://www.invivogen.com/bafilomycin-a1

For research use only

Version 23L08-MM

PRODUCT INFORMATION

Contents Bafilomycin A1 (BafA1) is available in two quantities:

- **tlrl-baf1:** 10 µg of Bafilomycin A1 - InvitroFit™
- **tlrl-baf1-10:** 10 x 10 µg of Bafilomycin A1 - InvitroFit™

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[1]. Therefore, it is frequently used to study functional autophagy.

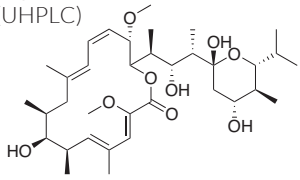
V-ATPases establish and maintain a low luminal pH in endocytic and exocytic compartments. Upon binding to the V-ATPase complex BafA1 inhibits H^+ translocation, thereby depriving acidic intracellular compartments (i.e. endosomes, lysosomes, and vesicles) of H^+ ions, increasing their pH and inhibiting 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[2]. 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[3].

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[3, 4]. 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[3].

1. Yamamoto A. *et al.*, 1998. Bafilomycin A1 prevents maturation of autophagic vacuoles by inhibiting fusion between autophagosomes and lysosomes in rat hepatoma cell line, H-4-II-E cells. Cell Struct Funct 23, 33-42. **2. Lee B.L. & Barton G.M., 2014.** Trafficking of endosomal Toll-like receptors. Trends Cell Biol. 24(6): 360–369. **3. Yan Y. *et al.*, 2016.** Bafilomycin A1 induces caspase-independent cell death in hepatocellular carcinoma cells via targeting of autophagy and MAPK pathways. Sci Rep 6, 37052. **4. Yuan N. *et al.*, 2015.** Bafilomycin A1 targets both autophagy and apoptosis pathways in pediatric B-cell acute lymphoblastic leukemia. Haematologica 100, 345-356.

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

1. Add 160 µl of DMSO to 10 µg of BafA1.
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-htlr9.

1. Add 20 µl of BafA1 (final concentration 100 nM to1 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% CO_2.
5. Determine inhibition by assessing SEAP expression using a SEAP detection medium, such as QUANTI-Blue™ Solution.

RELATED PRODUCTS

Product	Description	Cat.Code
HEK-Blue™ hTLR9 Cells	Reporter cells	hkb-htlr9
ODN 2006 (ODN 7909)	Human TLR9 agonist	tlrl-odn2006
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT
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