

Bafilomycin A1

Autophagy Inhibitor; V-ATPase inhibitor

Catalog code: tlr1-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 degradation¹. 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 pH². 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 cells³.

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 approach³.

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.

TECHNICAL SUPPORT

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CHEMICAL PROPERTIES

CAS number: 88899-55-2

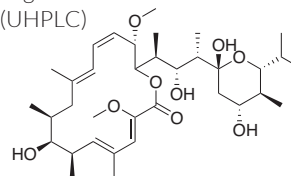
Formula: C₃₅H₅₈O₉

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-htrlr9>.

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

Product	Description	Cat.Code
HEK-Blue™ hTLR9 Cells	Reporter cells	hkb-htrlr9
ODN 2006 (ODN 7909)	Human TLR9 agonist	tlrl-odn2006
Wortmannin	Autophagy inhibitor	tlrl-wtm
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

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