

Brefeldin A

ER-Golgi protein trafficking inhibitor

Catalog Code: inh-bfa

<https://www.invivogen.com/bfa>

For research use only

Version 19H13-ED

PRODUCT INFORMATION

Contents

- 10 mg Brefeldin A

Storage and stability

- Brefeldin A is provided as an evaporated translucent film and shipped at room temperature. Upon receipt, store product at -20°C.
- Upon resuspension of Brefeldin A prepare aliquots and store at -20°C. Resuspended product is stable for up to 3 months when properly stored at -20°C.
- Avoid repeated freeze-thaw cycles.

Quality control

- Purity: ≥95% (UHPLC)
- Inhibition of the STING-induced IRF pathway by Brefeldin A has been confirmed using cellular assays.

PRODUCT DESCRIPTION

Brefeldin A (BFA) is a small hydrophobic macrocyclic lactone isolated from various soil and marine fungi¹. It is a potent and reversible inhibitor of the guanine nucleotide exchange factor GBF1. GBF1 is a key activator of ARF1p GTPase, which is essential for anterograde protein trafficking and vesicle formation between the endoplasmic reticulum (ER) and the Golgi apparatus¹. The blocking of vesicle trafficking in BFA-treated cells causes rapid accumulation of proteins in the ER, disrupting the intracellular trafficking of many proteins. Thus, BFA is commonly utilized as an inhibitor of protein secretion in cellular assays.

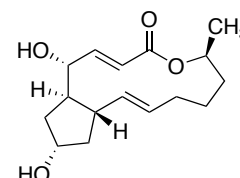
BFA also effectively inhibits the secretion of cytokines by blocking the trafficking of upstream signaling proteins. BFA inhibits type I interferon (IFN) production by blocking the dissociation of activated STING (stimulator of interferon genes) from the ER². This prevents the movement of STING to the ER-Golgi intermediate compartment, where it activates the TBK1-IRF3 signaling axis, and ultimately triggers expression of IFNs.

BFA and its analogs are promising inhibitors in drug development due to their apoptosis-inducing properties as well as other potent activities including antitumor, antifungal, and antiviral effects³. Interestingly, despite impairing NLRP3 inflammasome activation, BFA does not block the release of IL-1β, for which the secretion mechanism remains elusive⁴.

1. Chardin, P. & McCormick, F. 1999. Brefeldin A: the advantage of being uncompetitive. Cell 97, 153-155. 2. Dobbs, N. et al., 2015. STING Activation by Translocation from the ER Is Associated with Infection and Autoinflammatory Disease. Cell Host Microbe 18, 157-168. 3. Paek, S. M. 2018. Recent Synthesis and Discovery of Brefeldin A Analogs. Mar Drugs 16. 4. Hong, S. et al., 2019. Brefeldin A-sensitive ER-Golgi vesicle trafficking contributes to NLRP3-dependent caspase-1 activation. FASEB J 33, 4547-4558.

CHEMICAL PROPERTIES

- CAS Number: 20350-15-6
- Formula: C₁₆H₂₄O₄
- Molecular weight: 280.36 g/mol
- Solubility: 10 mg/ml DMSO



METHODS

Preparation of 20 mM stock solution (5.6 mg/ml)

1. Add 1.8 ml of DMSO to a single vial and vortex gently
2. Prepare aliquots and store at -20°C

Note: Further dilution to 10mM in DMSO may be required before diluting into the working concentration range with H₂O.

Working concentration range: 1 - 10 μM (for InvivoGen's cell-based assay)

Note: The working concentration of Brefeldin A will vary depending upon the application and will need to be optimized accordingly.

Inhibition of intracellular protein trafficking by BFA in cellular assays

Below is a protocol for using InvivoGen's THP1-Dual™ cells to study the inhibition of intracellular protein trafficking by BFA. These cells express both an inducible secreted embryonic alkaline phosphatase (SEAP) and an inducible Lucia luciferase to monitor the activation of the NF-κB and IRF (interferon regulatory factor) pathways, respectively. Changes in SEAP and Lucia luciferase expression levels due to ER-Golgi trafficking inhibition can be readily assessed using QUANTI-Blue™ Solution and QUANTI-Luc™ detection reagents, respectively.

Note: For the full description of the THP1-Dual™ cells, please visit <https://www.invivogen.com/thp1-dual>

1. Add 20 μl of BFA (10x conc) per well of a flat bottom 96-well plate.
2. Prepare a suspension of THP1-Dual™ cells (~900,000 cells per ml).
3. Add 160 μl of the cell suspension (~150,000 cells) to each well.
4. Incubate the plate at 37°C in a CO₂ incubator for 1 hour.
5. Add 20 μl of an inducer of the IRF pathway and/or the NF-κB pathway (e.g. 2'3'-cGAMP) and incubate the plate for 24 hours at 37°C in a CO₂ incubator
6. Prepare QUANTI-Luc™ (for IRF activation assessment) and/or QUANTI-Blue™ Solution (for NF-κB activation assessment) and carry out the measurement following the instructions on the data sheet.

RELATED PRODUCTS

Product	Cat. Code
2'3'-cGAMP	tlrl-nacga23-02
cAIM(PS)2 Difluor (Rp/Sp)	tlrl-nacairs
THP1-Dual™ cells	thpd-nfis
QUANTI-Blue™ Solution	rep-qbs
QUANTI-Luc™	rep-qlc1

TECHNICAL SUPPORT

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