# Brefeldin A

ER-Golgi protein trafficking inhibitor - InvitroFit™

Catalog Code: inh-bfa, inh-bfa-2

https://www.invivogen.com/bfa

For research use only

Version 23L07-MM

## PRODUCT INFORMATION

**Contents** Brefeldin A is provided as an evaporated translucent film and is available in two quantities:

- **inh-bfa**: 10 mg Brefeldin A - InvitroFit™
- **inh-bfa-2**: 20 mg (2 x 10 mg) Brefeldin A - InvitroFit™

**Storage and stability**

- Brefeldin A is 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.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

## PRODUCT DESCRIPTION

Brefeldin A (BFA) is a small hydrophobic macrocyclic lactone isolated from various soil and marine fungi[1]. 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 apparatus[1]. 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 ER[2]. 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 effects[3]. Interestingly, despite impairing NLRP3 inflammasome activation, BFA does not block the release of IL-1β, for which the secretion mechanism remains elusive[4].

**1. Chardin P. & McCormick F. 1999.** Brefeldin A: the advantage of being uncompetitive. Cell 97:153-5. **2. Dobbs N. *et al.*, 2015.** STING Activation by Translocation from the ER Is Associated with Infection and Autoinflammatory Disease. Cell Host Microbe 18:157-68. **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 33:4547-58.

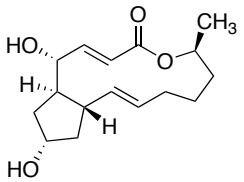
## CHEMICAL PROPERTIES

**CAS Number:** 20350-15-6

**Formula:** $C_{16}H_{24}O_4$

**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 $H_2O$.*

**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™ 4 Lucia/Gaussia 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 $CO_2$ 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 $CO_2$ incubator
6. Prepare QUANTI-Luc™ 4 Lucia/Gaussia (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 | Description | Cat. Code |
|---|---|---|
| 2'3'-cGAMP | STING ligand | tlrl-nacga23 |
| cAIM(PS)2 Difluor (Rp/Sp) | STING ligand | tlrl-nacairs-2 |
| THP1-Dual™ cells | Reporter monocytes | thpd-nfis |
| QUANTI-Blue™ Solution | SEAP detection medium | rep-qbs |
| QUANTI-Luc™ 4 Lucia/Gaussia | Luciferase detection medium | rep-qlc4lg1 |

TECHNICAL SUPPORT
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