Brefeldin A
ER-Golgi protein trafficking inhibitor
Catalog Code: inh-bfa
https://www.invivogen.com/bfa
For research use only
Version 19G30-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 HEK-Blue™ TLR2 and TLR4 cellular assays.
• Absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and TLR4 cellular assays.

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


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 - 10µM (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 luciferase. To monitor the activation of the NF-κB and IRF (interferon regulatory factor) pathways, respectively. Changes in SEAP and 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 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™ (for IRF activation assay) and/or QUANTI-Blue™ Solution (for NF-κB activation assay) and carry out the measurement following the instructions on the data sheet.

RELATED PRODUCTS

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. Code</th>
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<tbody>
<tr>
<td>2'3'-cGAMP</td>
<td>thr-nacga23-02</td>
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<tr>
<td>cAIM/PS12 Difluor (Rp/Sp)</td>
<td>thr-nacairs</td>
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<td>THP1-Dual™ cells</td>
<td>thpd-nifs</td>
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<tr>
<td>QUANTI-Blue™ Solution</td>
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<td>QUANTI-Luc™</td>
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