3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

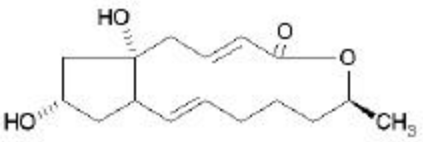
ProductInformation

Brefeldin A
from *Penicillium brefeldianum*
Molecular Biology Reagent

Product No. **B6542**
Storage Temperature 2-8 °C

CAS RN: 20350-15-6

Synonyms: Decumbin; Ascotoxin; Cyanein; BFA; γ,4-Dihydroxy-2-(6-hydroxy-1-heptenyl)-4-cyclo-pentanecrotonic acid λ-lactone



Molecular formula: $C_{16}H_{24}O_4$
Molecular weight: 280.36

Product Description
Brefeldin A (BFA) is a fungal metabolite produced by *Penicillium brefeldianum.* It is known to inhibit protein secretion in mammalian and other eukaryotic cells by interfering with the function of the Golgi apparatus. BFA may be used to study cell processes which depend upon intracellular protein transport. BFA is a potent inhibitor of intracellular transport of secretory proteins in rat hepatocytes. The drug specifically blocked the secretion, but caused no significant effect on protein synthesis.[1]

Brefeldin A has been reported to block the response of cultured cells to cholera toxin.[2] BFA inhibits protein synthesis in cultured cells[3] and inhibits the transport of secretory and lysosomal proteins at concentrations of 1-10 µg/mL.[4] "In HepG2 cells, BFA induces two blocks in the secretory pathway; one at the level of the endoplasmic reticulum-Golgi juncture and the other in the trans-Golgi network. In contrast, transport from the Golgi complex to the lysosomes and from the plasma membrane to the lysosomes continued." [4] Vogel, et al., also reported secretion blockage and redistribution of Golgi resident membrane proteins.[5] Lippincott-Schwartz, et al., reported on the effects of Brefeldin A (BFA) on the morphology and dynamics of endosomes, trans-Golgi network (TGN) and lysosomes. BFA treatment (at 5 mg/mL) induced changes in both the organization and distribution of the organellar components in all of these organellar systems.[6] Brefeldin A was reported to enhance transcytosis of transferrin in cultured kidney cells.[7]

The product is tested for its ability to inhibit IL-2 production by T-cells. T-cell activation is normally triggered by the interaction of a cell surface receptor to its specific ligand molecule. One of the cellular responses is the production and secretion of interleukin-2 (IL-2). Jurkat cells are a leukemic T-cell line known to produce IL-2. When Jurkat cells are stimulated by phorbol 12-myristate 13-acetate (PMA) and a co-stimulator, such as phytohemagglutinin (PHA), IL-2 production is strongly enhanced.[8] PHA by itself can trigger a low level of T-cell activation and IL-2 production by binding non-specifically to the cell surface receptor complex. The combination of PMA and PHA results in greatly increased IL-2 production and secretion. Brefeldin A blocks the secretory pathway by disrupting the movement of material from the endoplasmatic reticulum to the Golgi [1] apparatus.

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Sigma tests BFA for solubility in methanol at 10 mg/mL. Stock solutions of Brefeldin A can be prepared in methanol (1 mg/mL) [1] or in ethanol (5 mg/mL)[1,2] and stored at –20 °C.[2,9]
Concentration can be verified by UV absorption: λ_{max} = 215 nm, log E_M = 4.05 [10]

Storage/Stability
Store the product at 2-8 °C. Under these conditions the product is stable for 3 years. Methanol and ethanol solutions should be stored at –20 °C.

Product Summary
In the presence of 1 µg/ml PHA and 50 ng/ml PMA plus 10 µg/ml Brefeldin A, production of IL-2 was inhibited at least 90% that of control cells with PHA and PMA (without addition of Brefeldin A).

Suitability Assay
2.5 ml fresh culture medium was added to 25 cm^2 culture bottles. 2.5 ml of Jurkat cell culture (1 x 10^6 cells/ml) was added to each culture bottle. The following additions were made to duplicate bottles.

a. Control - no additions
b. 1 µg/ml PHA + 50 ng/ml PMA - add 10 µl PHA stock solution (0.5 mg/ml PHA in filter-sterilized PBS) + 2.5 µl PMA stock solution (100 µg/ml PMA in DMSO)
c. 10 µg/ml Brefeldin A + 1 µg/ml PHA + 50 ng/ml PMA - add 5 µl Brefeldin A stock solution (10 mg/ml Brefeldin A in absolute ethanol)+ 10 µl PHA stock solution + 2.5 µl PMA stock solution

The bottles were incubated at 37 °C for 24 hours. After centrifugation, the clarified broth was tested for IL-2 production by an ELISA assay. IL-2 production in the test cultures containing Brefeldin A was inhibited at least 90% compared to the PHA + PMA test cultures.

References:

1. Misumi, Y., et al., Novel blockade by brefeldin A of intracellular transport of secretory proteins in cultured rat hepatocytes., J. Biol. Chem., **261**, 11398-11403 (1986).
2. Orlandi, P.A., et al., Brefeldin A blocks the response of cultured cells to cholera toxin. Implications for intracellular trafficking in toxin action., J. Biol. Chem., **268**, 12010-12016 (1993).
3. Fishman, P.H. and Curran, P.K., Brefeldin A inhibits protein synthesis in cultured cells., FEBS Lett., **314**, 371-374 (1992).
4. Strous, G.J., et al., Differential effects of brefeldin A on transport of secretory and lysosomal proteins., J. Biol. Chem., **268**, 2341-2347 (1993).
5. Vogel, J.P. et al., Brefeldin A causes a defect in secretion in Saccharomyces cerevisiae., J. Biol. Chem., **268**, 3040-3043 (1993).
6. Lippincott-Schwartz, J., et al., Brefeldin A's effects on endosomes, lysosomes, and the TGN suggest a general mechanism for regulating organelle structure and membrane traffic., Cell, **67**, 601-616 (1991).
7. Wan, J. et al., Brefeldin A enhances receptor-mediated transcytosis of transferrin in filter-grown Madin-Darby canine kidney cells., J. Biol. Chem., **267**, 13446-13450 (1992).
8. Manger, B., et al., Differential effect of cyclosporin A on activation signaling in human T cell lines., J. Clin. Invest., 77, 1501-06 (1986).
9. Betina, V.,et al., Study of the antifungal activity of the antibiotic, cyanein..J. Antibiot., **17**, 93-95 (1964).
10. Merck Index, 12th ed., No. 1393 (1996).

NDH/PHC 12/04