Validation data for Brefeldin A

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Version 19G30-ED

Brefeldin A (BFA) is a small, potent, and reversible inhibitor of intracellular protein trafficking and vesicle formation between the endoplasmic reticulum (ER) and the Golgi apparatus. As ER-Golgi vesicle trafficking plays a pivotal role in the secretion of many proteins, BFA is commonly utilized as an inhibitor of protein secretion in cellular assays (**Figure 1**). Additionally, it has been established that BFA inhibits type I interferon (IFN) production by blocking the dissociation of activated STING from the ER (**Figure 2**). Of note, while morphological changes are seen in THP1 cells when BFA is used at concentrations above 0.6 µM, there is no change to the cellular viability.

Inhibition of STING-induced IRF activity by Brefeldin A

Treatment of THP1-Dual™ cells with BFA for 1 hour results in reduced levels of an IRF-inducible Lucia luciferase in a dose-dependent manner upon incubation with the STING ligands 2'3'-cGAMP and cAIM(PS)2 Difluor (Rp/Sp). Similiarly, reduced NF-κB-inducible SEAP (secreted alkaline phosphatase) is also observed in these cells (data not shown).

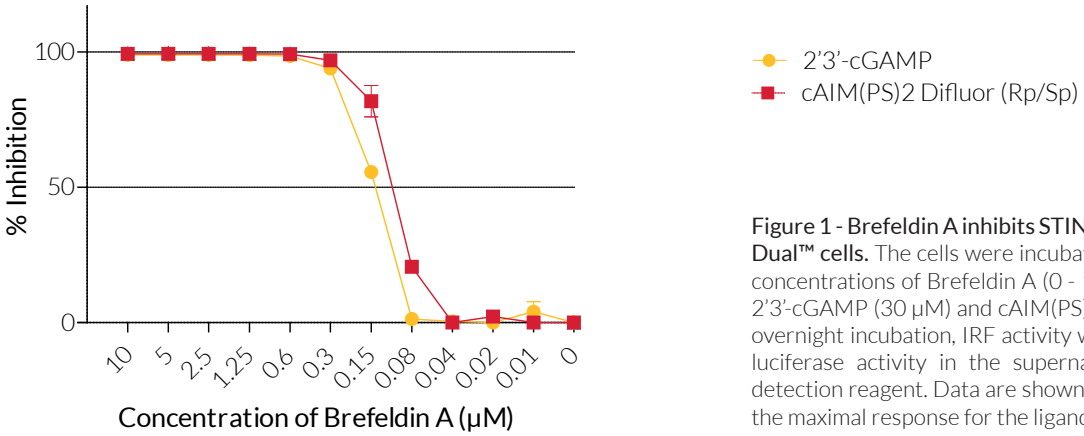

Figure 1 - Brefeldin A inhibits STING-induced IRF activity in THP1-Dual™ cells. The cells were incubated in the presence of increasing concentrations of Brefeldin A (0 - 10 µM) for 1 hour before adding 2'3'-cGAMP (30 µM) and cAIM(PS)2 Difluor (Rp/Sp) (10 µM). After overnight incubation, IRF activity was assessed by measuring Lucia luciferase activity in the supernatant, using the QUANTI-Luc™ detection reagent. Data are shown as a percentage (%) inhibition of the maximal response for the ligand with no BFA.

Inhibition of constitutive STING-induced IRF activity by Brefeldin A

BFA 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. Upon incubation with BFA for 18 hours, reduced levels of an IRF-inducible Lucia luciferase in a dose-dependent manner is observed in THP1-Dual™ KI-hSTING-S154 (constitutively active STING variant) cells. This cell line displays constitutive activation of STING and expression of an IRF-dependent Lucia luciferase in the absence of an inducer, due to the knock-in of a 'gain-of-function' mutation.

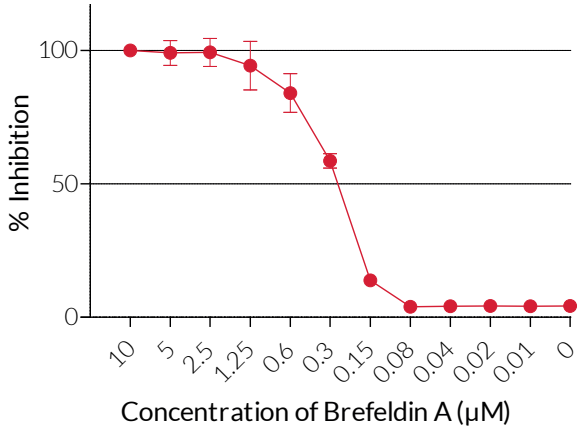

Figure 2 - Brefeldin A inhibits constitutively activated STING in THP1-Dual™ KI-hSTING-S154 cells. The cells were incubated in the presence of increasing concentrations of Brefeldin A (0 - 10 µM) for 18 hours. Inhibition of STING signaling was assessed by measuring the activity of the constitutively expressed Lucia luciferase in the supernatant, using the QUANTI-Luc™ detection reagent. Data are shown as a percentage (%) inhibition of the maximal response of the cell line with no BFA.

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