Validation data for Bafilomycin A1

https://www.invivogen.com/Bafilomycin A1

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Bafilomycin A1 (BafA1) is a specific vacuolar H⁺ ATPase (V-ATPase) inhibitor. Due to its ability to specifically target V-ATPase and hence disrupt autophagic flux, BafA1 is frequently used to study autophagy and endosomal acidification. Notably, by inhibiting V-ATPase, BafA1 can be used to block the activation of nucleic acid sensing endosomal Toll-Like receptors (TLRs), such as TLR9, by neutralizing endosomal pH.

The ability of BafA1 to inhibit V-ATPase was validated using InvivoGen's HEK-Blue $^{\text{IM}}$ hTLR9 cells. These cells were specifically designed for the study of human TLR9-induced NF- $^{\text{IK}}$ B signaling by monitoring the activity of secreted embryonic alkaline phosphatase (SEAP) reporter activity. The SEAP production by these cells was measured using QUANTI-Blue $^{\text{IM}}$ Solution, a SEAP detection reagent. Treatment with BafA1 inhibited SEAP activity in a dose-dependent manner (Figure 1).

Dose-dependent V-ATPase inhibition

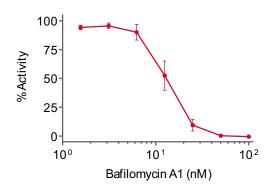


Figure 1: Bafilomycin A1 inhibits the TLR9 response in a dose-dependent manner.

HEK-Blue^{\pm} hTLR9 cells were stimulated with ODN 2006 (0.3 µg/ml) and increasing concentrations of BafA1. After overnight incubation, BafA1-induced inhibition of TLR9-NF- \pm B signaling was assessed by measuring the levels of SEAP using QUANTI-Blue^{\pm} Solution and by reading the optical density (OD) at 630 nm. Data are shown as percentage (%) activity \pm SEM.



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