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™ hTLR9 cells. These cells were specifically designed for the study of human TLR9-induced NF-κB signaling by monitoring the activity of secreted embryonic alkaline phosphatase (SEAP) reporter activity. The SEAP production by these cells was measured using QUANTI-Blue™ Solution, a SEAP detection reagent. Treatment with BafA1 inhibited SEAP activity in a dose-dependent manner (Figure 1).

![Dose-dependent V-ATPase inhibition](image)

**Figure 1:** Bafilomycin A1 inhibits the TLR9 response in a dose-dependent manner.
HEK-Blue™ 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-κB signaling was assessed by measuring the levels of SEAP using QUANTI-Blue™ Solution and by reading the optical density (OD) at 630 nm. Data are shown as percentage (%) activity ± SEM.