

Validation data for ODN TTAGGG (A151)

<https://www.invivogen.com/odnttaggg>

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Version 21L20-MM

ODN TTAGGG (A151) is a synthetic oligonucleotide (ODN) containing 4 repeats of the immunosuppressive TTAGGG motif. This product is a TLR9 antagonist that inhibits TLR9 activation by CpG-containing ODNs. This ODN was tested with InvivoGen's HEK-Blue™ hTLR9 reporter cells which stably express human TLR9 and a SEAP (secreted embryonic alkaline phosphatase) reporter gene. In these cells, ODN TTAGGG (A151) elicits a dose-dependent inhibition of CpG ODN-induced NF-κB activation (Figure 1). In addition, it functions as an inhibitor of the AIM2 inflammasome. When compared to other inflammasome inhibitors, ODN TTAGGG (A151) exhibits potent and specific inhibition of AIM2 inflammasome signaling (Figure 2). ODN TTAGGG (A151) has also been reported as an inhibitor of the cytosolic DNA sensor (CDS) cGAS. When tested with InvivoGen's THP1-Dual™ reporter cells which express multiple CDSs and two inducible reporter genes (interferon regulatory factor (IRF)-inducible Lucia luciferase and NF-κB-inducible SEAP), ODN TTAGGG (A151) exhibits a dose-dependent inhibition of IRF and NF-κB induction by cytosolic nucleic acids (Figure 3).

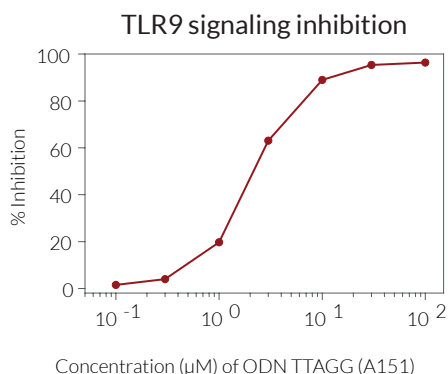


Figure 1. Dose-dependent inhibition of TLR9 activity in HEK-Blue™ hTLR9 cells. Cells were treated with the human CpG-ODN 2006 (1 µg/ml) in the presence of increasing concentrations of ODN TTAGGG (A151). After overnight incubation, the NF-κB response was determined using QUANTI-Blue™ Solution, a SEAP detection reagent. Data represent % inhibition of the signal.

Specific AIM2 inflammasome signaling inhibition

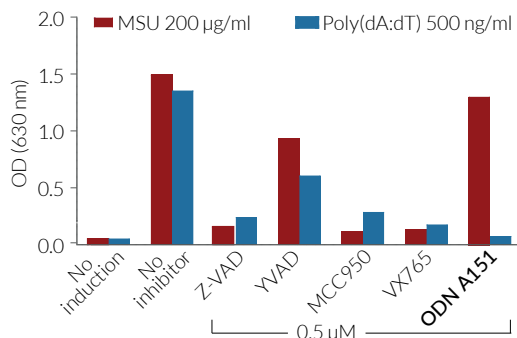


Figure 2. Effect of inflammasome inhibitors on IL-1β release by THP1-HMGB1-Lucia™ cells. Cells were primed with LPS (1 µg/ml) for 3 hours prior to treatment with 200 µg/ml MSU (NLRP3 inflammasome inducer) or 0.5 µg/ml Poly(dA:dT) complexes (AIM2 inflammasome inducer) in the presence of 0.5 µM Z-VAD (pan-caspase inhibitor), Ac-YVAD-cmk (caspase-1 inhibitor), MCC950 (NLRP3 inflammasome inhibitor), VX-765 (caspase-1 inhibitor) and ODN TTAGGG (A151; AIM2 inhibitor). After overnight incubation, cell supernatants were added to HEK-Blue™ IL-1β cells for 16 hours. IL-1β levels were determined by assessing SEAP activity in the supernatant using QUANTI-Blue™ Solution.

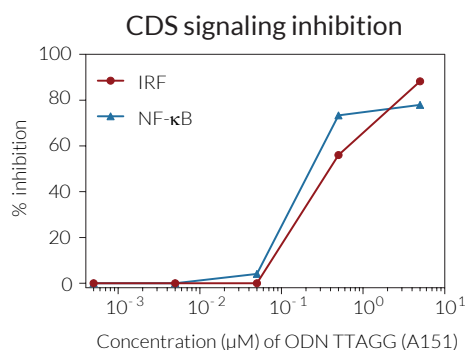


Figure 3. Inhibition of CDS activity in THP1-Dual™ cells. Cells were pre-treated with increasing concentrations of ODN TTAGGG (A151) for 6 hours followed by stimulation with cytosolic double-stranded DNA (1 µg/ml). Prior to experimentation, the DNA was complexed with a transfection reagent to facilitate intracellular delivery. After overnight incubation, the IRF and NF-κB responses were determined using QUANTI-Luc™ and QUANTI-Blue™ Solution, respectively.

TECHNICAL SUPPORT

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