Validation data for Alum Hydroxide

https://www.invivogen.com/alum

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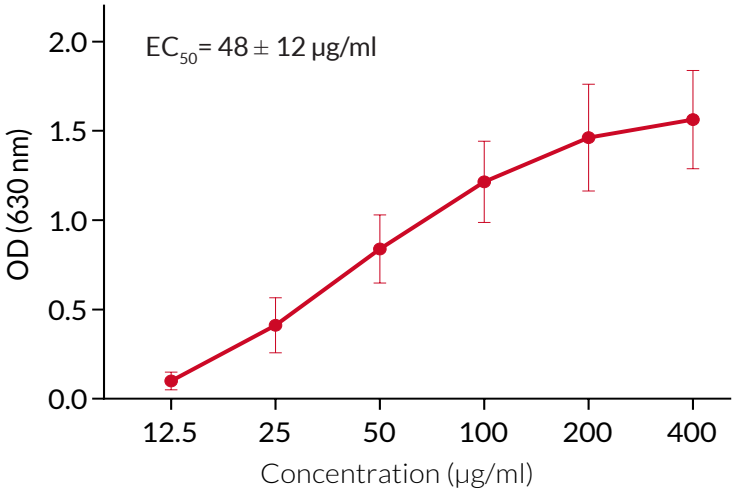
Version 19L06-MM

Aluminium Hydroxide (alum) is an inducer of the NLRP3 inflammasome, a large intracellular multiprotein complex that plays a central role in innate immunity[1-2]. NLRP3 inflammasome activation requires an initial signal ('priming'), provided by microbial molecules, such as lipopolysaccharide (LPS), and a secondary signal, provided by a wide array of stimuli including bacterial toxins, endogenous molecules or crystalline substances such as alum. This triggers the multimerization of the NLRP3 inflammasome and caspase-1 activation with the subsequent maturation and secretion of IL-1β and IL-18.

InvivoGen's Alum Hydroxide is designed for *in vitro* assays. Its ability to induce the NLRP3 inflammasome has been validated using THP1-Null cells. The production of IL-1β by THP1-Null cells was measured using HEK-Blue™ IL-1β cells. Treatment with Alum Hydroxide induced IL-1β secretion, an indicator of NLRP3 inflammasome activation, in a dose-dependent manner.

1. Schroder K. & Tschopp J., 2010. The inflammasomes. Cell 140(6):821-32. **2. Franchi L. *et al.*, 2012.** Sensing and reacting to microbes through the inflammasomes. Nat Immunol 13(4)325-32.

Evaluation of NLRP3 inflammasome activation



IL-1β production in THP1-Null cells. THP1-Null cells, primed with LPS (1 µg/ml for 3h), were stimulated with increasing concentrations of Alum Hydroxide. After overnight incubation, IL-1β secretion was analyzed by adding 50 µl of supernatant from treated THP1-Null cells to HEK-Blue™ IL-1β cells. IL-1β-induced activation of NF-κB was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1β cells using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 630 nm.

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