

# Validation data for CPPD crystals

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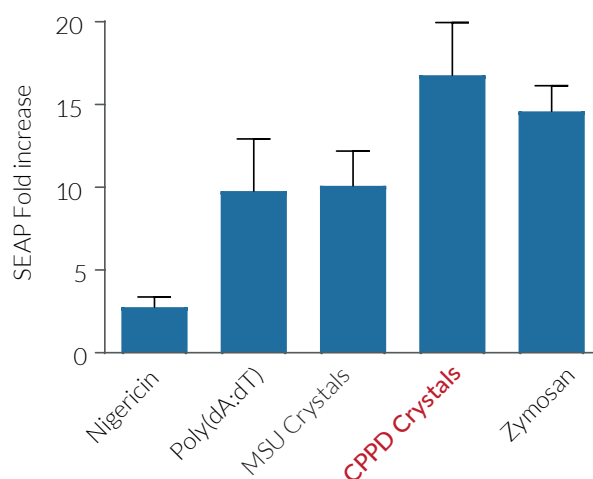
Version 21L22-MM

Calcium pyrophosphate dihydrate (CPPD) crystals are inducers of the NLRP3 inflammasome, a large intracellular multiprotein complex that plays a central role in innate immunity<sup>1,2</sup>. 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 structures such as CPPD crystals. This triggers the multimerization of the NLRP3 inflammasome and caspase-1 activation with the subsequent maturation and secretion of IL-1 $\beta$  and IL-18.

The ability of CPPD crystals to induce the NLRP3 inflammasome has been validated using THP1-HMGB1-Lucia™ cells. The production of IL-1 $\beta$  by THP1-HMGB1-Lucia™ cells was measured using HEK-Blue™ IL-1 $\beta$  cells. Treatment with CPPD crystals induced IL-1 $\beta$  secretion, an indicator of NLRP3 inflammasome activation.

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 inflammasome activation



**Figure 1. IL-1 $\beta$  production in THP1-HMGB1-Lucia™ cells.** Cells were primed with 1  $\mu$ g/ml LPS-EK for 3h and then incubated with inflammasome inducers; 8  $\mu$ M Nigericin, 0.5  $\mu$ g/ml complexed Poly(dA:dT), 200  $\mu$ g/ml MSU Crystals, 100  $\mu$ g/ml CPPD Crystals, or 1 mg/ml Zymosan. After 24h, supernatants were incubated with HEK-Blue™ IL-1 $\beta$  cells overnight and SEAP activity was assessed using QUANTI-Blue™ Solution. The optical density (OD) was read at 630 nm. Data are shown as a fold increase (mean  $\pm$  SEM) over non-induced cells.

### TECHNICAL SUPPORT

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