

# CPPD Crystals

## NLRP3 inflammasome Inducer

Catalog # tlr1-cppd

For research use only

Version # 11C13-MM

### PRODUCT INFORMATION

#### Content:

- 5 mg calcium pyrophosphate dihydrate (CPPD) crystals

#### Storage and stability:

- CPPD crystals are shipped at room temperature. Store at 4°C.
- Upon resuspension, CPPD crystals should be stored at 4°C for 6 months or -20°C for 1 year.

### DESCRIPTION

Calcium pyrophosphate dihydrate (CPPD) is the aetiological agent of the joint acute inflammatory disease pseudogout. Similarly to uric acid, the causative agent of gout, CPPD crystals can act as endogenous danger signals that stimulate the innate immune system to produce inflammatory cytokines such as interleukin (IL)-1 $\beta$ . Recently, CPPD crystals were shown to activate caspase-1, which is required for IL-1 $\beta$  maturation, through the NLRP3 (NALP3) inflammasome<sup>2</sup>. The NLRP3 inflammasome is a caspase-1-activating complex comprising the NLR protein NLRP3 and the adaptor ASC<sup>3</sup>. Engagement of the NLRP3 inflammasome is supported by the finding that macrophages from mice deficient in various components of the inflammasome do not respond to injection of CPPD crystals<sup>2</sup>.

1. Shi *et al.*, 2003. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature* 425(6957):516-21
2. Martinon F. *et al.*, 2006. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 440(7081):237-41.
3. Martinon F, Tschopp J., 2004. Inflammatory caspases: linking an intracellular innate immune system to autoinflammatory diseases. *Cell*. 117(5):561-74.

### CHEMICAL PROPERTIES

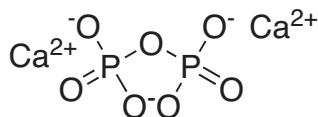
Linear formula: Ca<sub>2</sub>O<sub>7</sub>P<sub>2</sub> 2H<sub>2</sub>O

Molecular weight: 254.1

Structure: Triclinic crystals characterized by X-ray diffraction

Appearance: White powder

Solubility: Not soluble



### METHODS

#### Preparation of 5 mg/ml CPPD stock solution

- Add 1 ml of PBS to the 5 mg CPPD vial.

*Note:* CPPD is not soluble. Vortex or sonicate (for 5 minutes) prior to each use to obtain a homogenous suspension.

- Prepare further dilutions by adding the appropriate amount of PBS.

#### Detection of NLRP3 inflammasome induction

Secretion of IL-1 $\beta$  is an indicator of the NLRP3 inflammasome induction. The activation and release of IL-1 $\beta$  requires two distinct signals: the first signal leads to the transcriptional upregulation and synthesis of pro-IL-1 $\beta$ ; the second signal leads to IL-1 $\beta$  maturation and secretion through the activation of NLRP3 inflammasome.

The synthesis of pro-IL-1 $\beta$  can be induced by priming human monocytic THP-1 cells for 3 h with PMA (phorbol 12-myristate 13-acetate; 300 ng/ml) or LPS (lipopolysaccharide, 1  $\mu$ g/ml). Subsequent stimulation with 50 - 200  $\mu$ g/ml CPPD leads to the formation of NLRP3 inflammasome resulting in IL-1 $\beta$  maturation and secretion. Secreted IL-1 $\beta$  can be detected by Western blot or ELISA. Alternatively, InvivoGen recommends the use of HEK-Blue™ IL-1 $\beta$  cells, a reporter cell line that specifically detects bioactive IL-1 $\beta$ . These cells express an NF- $\kappa$ B and AP-1-inducible SEAP (secreted alkaline phosphatase) reporter gene. The presence of IL-1 $\beta$  leads to NF- $\kappa$ B and AP-1 activation and the subsequent secretion of SEAP. Levels of SEAP can be easily determined with HEK-Blue™ Detection or QUANTI-Blue™, detection media that turn purple/blue in the presence of alkaline phosphatase. HEK-Blue™ Detection is designed for high-throughput detection of SEAP, while QUANTI-Blue™ is more sensitive and designed for the detection and quantification of SEAP.

### RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ IL-1 $\beta$ Cells	hkb-il1b
<i>E.coli</i> K12 LPS	tlrl-eklps
Other inflammasome inducers:	
Alum crystals	tlrl-alk
ATP	tlrl-atp
Hemozoin	tlrl-hz
MSU crystals	tlrl-msu
Nigericin	tlrl-nig
Poly(dA:dT)	tlrl-pat

#### TECHNICAL SUPPORT

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