CPPD Crystals

NLRP3 inflammasome inducer

Catalog code: tlrl-cppd

https://www.invivogen.com/cppd-crystals

For research use only

Version 21I21-MM

PRODUCT INFORMATION

Contents

• 5 mg of calcium pyrophosphate dihydrate (CPPD) crystals

Storage and stability

- CPPD crystals are shipped at room temperature. Upon receipt, store at 4°C.
- Upon resuspension, store at 4°C or at -20°C. Resuspended CPPD crystals are stable for 6 months at 4°C and for 1 year at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- The biological activity has been confirmed using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

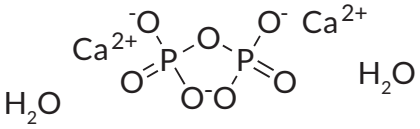
DESCRIPTION

Calcium pyrophosphate dihydrate (CPPD) is the aetiological agent of the joint acute inflammatory disease pseudogout. Similar 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)-1β[1]. CPPD crystals were shown to activate caspase-1, which is required for IL-1β maturation, through the NRLP3 (NALP3) inflammasome[2]. The NLRP3 inflammasome is a caspase-1-activating complex comprising the NLR protein NLRP3 and the adaptor ASC[3]. Engagement of the NRLP3 inflammasome is supported by the finding that macrophages from mice deficient in various components of the inflammasome do not respond to the injection of CPPD crystals[2].

1. Shi Y. *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

CAS Number: 7790-76-3
Linear formula: $Ca_2O_7P_2 \cdot 2H_2O$
Molecular weight: 254.1 g/mol
Solubility: Insoluble
Structure: Triclinic crystals characterized by X-ray diffraction



METHODS

Preparation of CPPD stock suspension (5 mg/ml)

• Add 1 ml of sterile phosphate buffered saline (PBS; not provided) to 5 mg of CPPD crystals.

Note: CPPD crystals are not soluble in aqueous solutions. Vortex or sonicate (for 5 minutes) prior to each use to obtain a homogenous suspension.

• Prepare further dilutions by adding the appropriate amount of sterile PBS.

NLRP3 INFLAMMASOME INDUCTION

CPPD crystals can be used to induce the NLRP3 inflammasome in cellular assays, such as InvivoGen's THP-1/HEK-Blue™ IL-1β assay. This assay uses the secretion of IL-1β by THP1-Null2 cells as an indicator of NLRP3 inflammasome induction. The IL-1β production by these cells is measured using HEK-Blue™ IL-1β cells.
For more information, visit https://www.invivogen.com/thp1-nullz.

Production of IL-1β by THP1-Null2 cells

1. Prepare a THP1-Null2 cell suspension at 1.6 x 10^6 cells/ml and add 180 µl of this cell suspension per well of a 96-well plate (~300,000 cells/well).
2. Prime THP1-Null2 cells with 20 µl of lipopolysaccharide (LPS; final concentration 1 µg/ml) for 3 hours at 37 °C in 5% CO_2.
3. Carefully remove medium and add 180 µl of supplemented RPMI.
4. Add 20 µl of CPPD crystals (1-50 µg/ml final concentration).
5. Incubate overnight at 37 °C in 5% CO_2.

Detection of IL-1β

Secreted IL-1β from the supernatant of the treated THP1-Null2 cells can be detected using InvivoGen's HEK-Blue™ IL-1β cells. For more information, visit https://www.invivogen.com/hek-blue-il1b.

RELATED PRODUCTS

Product	Description	Cat. Code
Alum Hydroxide	Inflammasome inducer	tlrl-aloh
ATP	Inflammasome inducer	tlrl-atpl
HEK-Blue™ IL-1β Cells	IL-1β reporter cells	hkb-il1bv2
Hemozoin	Inflammasome inducer	tlrl-hz
LPS-EK	LPS from *E. coli* K12	tlrl-eklps
MSU crystals	Inflammasome inducer	tlrl-msu
Nigericin	Inflammasome inducer	tlrl-nig
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
THP1-Null2 cells	THP-1-derived monocytes	thp-nullz

TECHNICAL SUPPORT
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