ATP NLRP3 inflammasome inducer Catalog code: tlrl-atpl https://www.invivogen.com/atp

> For research use only Version 20K18-MM

PRODUCT INFORMATION

Contents

- 1 g ATP (adenosine 5'-triphosphate disodium salt) provided lyophilized

Storage and stability

- ATP is shipped at room temperature. Store at -20 °C.

- Upon resuspension, prepare aliquots of ATP and store at -20 °C. Resuspended product is stable for 6 months when properly stored.

Quality control

Purity: ≥99.0% (HPLC)
The biological activity of ATP has been confirmed using the

inflammasome induction assay based on THP1-Null cells and HEK-Blue^{\sim} IL-1 β cells.

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

DESCRIPTION

Adenosine triphosphate (ATP), a potassium efflux agent, can trigger the activation of NALP3 inflammasome in response to PAMPs, such as lipopolysacchairde (LPS) and peptidoglycan. It stimulates the caspase-1-dependent cleavage and secretion of IL-1 β from LPS-stimulated cells³. ATP triggers the opening of the non-selective cation channel of the purinergic P2X7 receptor, followed by the gradual opening of a larger pore. The larger pore is attributed to pannexin-1, which is recruited upon P2X7 receptor activation⁴. Activation of the P2X7 receptor results in potassium efflux which is necessary for activation of the post-translational maturation of IL-1 β ⁵.

1. Mariathasan S. *et al.*, 2006. Cryopyrin activates the inflammasome and ATP. Nature 440;228-32. 2. Locovei S. *et al.*, 2007. Pannexin1 is part of the pore forming unit of the P2X(7) receptor death complex. FEBS Lett. 581(3):483-8. 3. Perregaux D. & Gabel CA., 1994. Interleukin-1b maturation and release in response to ATP and nigericin. J Biol. Chem. 269:15195-15203.

CHEMICAL PROPERTIES

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TECHNICAL SUPPORT

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METHODS

Preparation of stock solution (200 mM)

1. Add 9.072 ml of endotoxin-free water to 1 g of ATP.

2. Vortex until completely dissolved.

<u>Note:</u> The ATP stock solution is acidic. The pH of this solution can be adjusted using 4 M NaOH.

3. Further dilutions can be prepared using the appropriate buffers.

Detection of NLRP3 inflammasome induction

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

The synthesis of pro-IL-1 β can be induced by priming human monocytic THP-1 cells for 3 h with PMA (phorbol 12-myristate 13-acetate; 20-50 ng/ml) or LPS (lipopolysaccharide, 1 µg/ml). Subsequent stimulation with 5 mM ATP leads to the formation of NLRP3 inflammasome resulting in IL-1 β maturation and secretion. Secreted IL-1 β can be detected by Western blot or ELISA. Alternatively, InvivoGen recommends the use of HEK-Blue" IL-1b cells, a reporter cell line that specifically detects bioactive IL-1 β . These cells express an NF-kB and AP-1-inducible SEAP (secreted alkaline phosphatase) reporter gene. The presence of IL-1 β leads to NF-kB and AP-1 activation and the subsequent secretion of SEAP. Levels of SEAP can be easily determined with QUANTI-Blue", a SEAP detection reagent that turn purple/blue in the presence of alkaline phosphatase. For more information, visit: https://www.invivogen.com/inflammasome-test-cells.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue [™] IL-1β Cells LPS-EK (LPS from <i>E. coli</i> K12) PMA THP1-Null Cells	hkb-il1b tlrl-eklps tlrl-pma thp-null
Other NLRP3 inflammasome inducers: Alum Hydroxide CPPD crystals Hemozoin MSU crystals Nano-SiO ₂ Nigericin TDB (Trehalose-6,6-dibehenate)	tlrl-aloh tlrl-cppd tlrl-hz tlrl-msu tlrl-sio tlrl-nig tlrl-tdb

