ADP-Heptose

ALPK1/TIFA agonist

Catalog code: tlrl-adph

https://www.invivogen.com/adp-heptose

For research use only

Version 20H17-ED

PRODUCT INFORMATION

Contents

•250 µg ADP-Heptose

Storage and stability

- ADP-Heptose is provided as a dried powder and shipped at room temperature. Upon receipt, store product at -20 °C.
- Upon resuspension of ADP-Heptose, prepare aliquots and store at -20 °C. Resuspended product is stable for up to 3 months when properly stored.
- Avoid repeated freeze-thaw cycles.

Quality control

- Purity: ≥95% (UHPLC)
- Activation of the ALPK1/TIFA signaling pathway has been confirmed using cellular assays.
- Absence of bacterial contamination (i.e. endotoxins) has been confirmed using a kinetic chromogenic LAL assay, with an endotoxin level <1 EU/mg.

PRODUCT DESCRIPTION

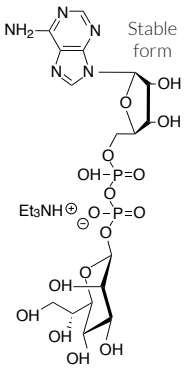
InvivoGen has synthesized and purified a stable form of ADP-L-*glycero*-b-D-*manno*-heptose (ADP-Heptose), an intermediary sugar in the biosynthesis of lipopolysaccharide (LPS), an essential component of the Gram-negative bacterial outer membrane.

ADP-L, D-Heptose is generated by a multi-step biosynthetic pathway, and is the precursor to activated L,D-heptose units, which are ultimately integrated into the inner core region of LPS[1]. ADP-Heptose has been identified as a potent pathogen associated molecular pattern (PAMP) of Gram-negative bacteria (e.g. *Helicobacter pylori*, and *Shigella flexneri*) that binds to the cytosolic pattern recognition receptor (PRR) ALPK1[1-3]. By binding to ALPK1, ADP-Heptose triggers the oligomerization of TIFA (TRAF-interacting protein with FHA domain-containing protein A) and the recruitment of TRAF6 (TNF receptor-associated factor 6). Ultimately, resulting in the activation of NF-κB and a strong pro-inflammatory response[1].

It has been shown that ADP-Heptose can be delivered to host cells (e.g. human embryonic kidney cells, intestinal epithelial cells, gastric cells, and cervical cancer cells) via the type III and type IV bacterial secretion systems of *Yersinia pseudotuberculosis* and *H. pylori*, respectively[1,3]. Additionally, extracellular ADP-Heptose can freely penetrate the host cell membrane and access the host cytoplasm. Thus, extracellular bacteria that do not encode these secretion systems (e.g. *Neisseria meningitidis*) are also able to activate the ALPK1-TIFA signaling axis[1].

1. Pfannkuch, L. *et al.* 2019. ADP heptose, a novel pathogen-associated molecular pattern identified in Helicobacter pylori. FASEB J, fj201802555R. **2. Garcia-Weber, D. *et al.* 2018.** ADP-heptose is a newly identified pathogen-associated molecular pattern of Shigella flexneri. EMBO Rep 19 **3. Zhou, P. *et al.* 2018.** Alpha-kinase 1 is a cytosolic innate immune receptor for bacterial ADP-heptose. Nature 561, 122-126.

CHEMICAL PROPERTIES



- Formula: $C_{17}H_{27}N_5O_{16}P_2.Et_3NH$ (stable form)
- Molecular weight: 720.21 g/mol (stable form)
- Solubility: 10 mg/ml H_2O

METHODS

Preparation of stock solution (1 mg/ml)

1. Add 250 µl of sterile H_2O and vortex until completely resuspended.
2. Prepare aliquots and store at -20 °C

Working concentration range:

0.01 - 30 µg/ml

Activation of the ALPK1-TIFA signaling axis by ADP-Heptose

Below is a protocol for using InvivoGen's HEK-Blue™ Null1-v cells for studying the activation of the ALPK1-TIFA signaling pathway. These cells express an inducible secreted embryonic alkaline phosphatase (SEAP) to monitor the activation of NF-κB. Changes to the expression levels can be readily assessed using QUANTI-Blue™ Solution, a SEAP detection reagent. Additionally, we recommend to use InvivoGen's HEK-Blue™ KO-TIFA and HEK-Blue™ KO-ALPK1 cells as negative control cell lines.

Note: For the full description of the HEK-Blue™ Null1-v, HEK-Blue™ KO-TIFA and HEK-Blue™ KO-ALPK1 cells, please visit https://www.invivogen.com/ko-alpk1-tifa-cells

1. Add 20 µl ADP-Heptose (0.01 - 30 µg/ml final concentration) per well of a flat bottom 96-well plate.
2. Prepare a suspension of HEK-Blue™ Null1-v cells (~280,000 cells per ml). As a negative control, prepare a suspension of HEK-Blue™ KO-TIFA and/or HEK-Blue™ KO-ALPK1 cells (~280,000 cells per ml).
3. Add 180 µl of the cell suspension (~50,000 cells) to the wells.
4. Add 180 µl of the negative control cell suspension (~50,000 cells) to another well.
5. Incubate the plate at 37°C in a CO_2 incubator for 20-24 h.
6. Prepare QUANTI-Blue™ Solution (for NF-κB activation assessment) and carry out the measurement following the instructions on the data sheet.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ Null1-v	Human NF-κB reporter cells	hkb-null1v
HEK-Blue™ KO-TIFA	TIFA knock out reporter cells	hkb-kotifa
HEK-Blue™ KO-ALPK1	ALPK1 knockout reporter cells	hkb-koalpk1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

TECHNICAL SUPPORT

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