ADP-L-Heptose

ALPK1/TIFA agonist; ADP-L-glycero-β-D-manno-heptose

Catalog code: tlrl-adph-l

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

For research use only

Version 21F07-ED

PRODUCT INFORMATION

Contents

- 250 µg **ADP-L-Heptose**
- 1.5 ml endotoxin-free water

Storage and stability

- ADP-L-Heptose is provided as a dried powder and shipped at room temperature. Upon receipt, store product at -20 °C.
- Upon resuspension of ADP-L-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

Bacterial ADP-Heptose is an intermediary sugar in the biosynthesis of lipopolysaccharide (LPS), an essential component of the Gram negative bacterial outer membrane. It is generated by a multi-step biosynthesis pathway, in which the final step is the interconversion between two isomers, ADP-D-glycero-β-D-manno-heptose and ADP-L-glycero-β-D-manno-heptose, catalyzed by an epimerase enzyme (e.g. HldD). InvivoGen has synthesized and purified a stable form of ADP-**L**-glycero-β-D-manno-heptose (L-isomer; **ADP-L-Heptose**).

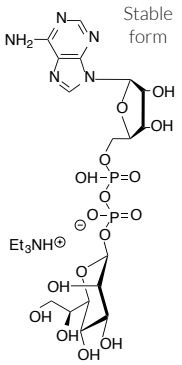
ADP-L-Heptose has been identified as a potent PAMP of Gram-negative bacteria (e.g. *Helicobacter pylori* and *Shigella flexneri*) that binds to the cytosolic ALPK1 receptor[1-3]. By binding to ALPK1, ADP-L-Heptose triggers the oligomerization of TIFA and the recruitment of TRAF6. Ultimately, resulting in the activation of NF-κB and a strong pro-inflammatory response[1-3].

It has been shown that ADP-L-Heptose can be delivered to host cells (e.g. human embryonic kidney cells) via the type III and type IV bacterial secretion systems of *Yersinia pseudotuberculosis* and *H. pylori*, respectively[1,3]. Additionally, extracellular ADP-L-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[1-3].

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-L-Heptose

Below is a protocol for using InvivoGen's HEK-Blue™ Null1-v cells, together with HEK-Blue™ KO-TIFA and/or HEK-Blue™ KO-ALPK1 cells to study the activation of the ALPK1-TIFA signaling pathway in response to ADP-L-Heptose.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 the SEAP detection reagent QUANTI-Blue™ Solution.

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-L-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 specificity 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 specificity 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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