

# ADP-Heptose

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

Catalog code: tlr1-adph

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

For research use only

Version 21F08-ED

## PRODUCT INFORMATION

### Contents

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

### 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

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-D-glycero-β-D-manno-heptose (D-isomer; **ADP-Heptose**).

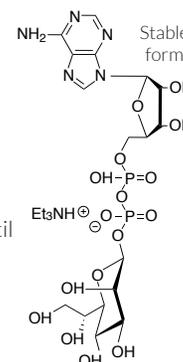
ADP-Heptose has been identified as a potent pathogen associated molecular pattern (PAMP) of Gram-negative bacteria (e.g. *Helicobacter pylori* and *Shigella flexneri*) that binds to the cytosolic PRR, ALPK1<sup>1-3</sup>. By binding to ALPK1, ADP-Heptose triggers the oligomerization of TIFA (TRAF-interacting protein with FHA domain-containing protein A) and the recruitment of TRAF6. Ultimately, resulting in the activation of NF-κB and a strong pro-inflammatory response<sup>1-3</sup>.

It has been shown that ADP-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<sup>1-3</sup>. 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<sup>1-3</sup>.

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<sub>17</sub>H<sub>27</sub>N<sub>5</sub>O<sub>16</sub>P<sub>2</sub>·Et<sub>3</sub>NH (stable form)
- Molecular weight: 720.21 g/mol (stable form)
- Solubility: 10 mg/ml H<sub>2</sub>O



## METHODS

### Preparation of stock solution (1 mg/ml)

1. Add 250 µl of sterile H<sub>2</sub>O 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**, 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-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-alkp1-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 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 **negative control** cell suspension (~50,000 cells) to another well.
5. Incubate the plate at 37°C in a CO<sub>2</sub> 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
ADP-L-Heptose	ALPK1 Ligand (L isomer)	tlr1-adph-l
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-koalkp1
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

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www.invivogen.com