C12-iE-DAP

NOD1 ligand

Catalog code: tlrl-c12dap

https://www.invivogen.com/c12-ie-dap

For research use only

Version 21C31-MM

PRODUCT INFORMATION

Contents:

• 1 mg C12-iE-DAP. This product is chemically synthesized.

Note: C12-iE-DAP is a mixture of Lauroyl-γ-D-Glu-D-mDAP and Lauroyl-γ-D-Glu-L-mDAP.

• 1.5 ml endotoxin-free water

Storage and stability:

- C12-iE-DAP is provided lyophilized and shipped at room temperature. Upon receipt, store at -20°C.

- Upon resuspension, prepare aliquots of C12-iE-DAP and store at -20°C. Resuspended product is stable for 1 year when properly stored. Avoid repeated freeze-thaw cycles.

Quality control:

- The NOD1 biological activity has been validated using HEK-Blue™ NOD1 cells.

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

DESCRIPTION

C12-iE-DAP is an acylated derivative of the dipeptide iE-DAP (γ-D-Glu-mDAP), present in the peptidoglycan of a subset of bacteria that include Gram-negative bacilli and some Gram-positive bacteria such as *Bacillus subtilis* and *Listeria monocytogenes*[1]. C12-iE-DAP was generated by addition of a lauroyl (C12) group to the glutamic residue of iE-DAP. Similarly to iE-DAP, C12-iE-DAP is recognized by NOD1 (CARD4), an intracellular sensor expressed in multiple tissues, including intestinal epithelia cells. Recognition of C12-iE-DAP by NOD1 induces a signaling cascade involving the serine/threonine RIP2 (RICK, CARDIAK) kinase, which interacts with IKK to trigger the activation of NF-κB and the production of inflammatory cytokines such as TNF-α and IL-6[2]. C12-iE-DAP stimulates NOD1 at concentrations 100- to 1000-fold lower than does iE-DAP.

1. Chamaillard M. *et al.*, 2003. An essential role for NOD1 in host recognition of bacterial peptidoglycan containing diaminopimelic acid. Nat. Immunol.4(7):702-7.
2. Park JH. *et al.*, 2007. RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs. J Immunol. 178(4):2380-6.

CHEMICAL PROPERTIES

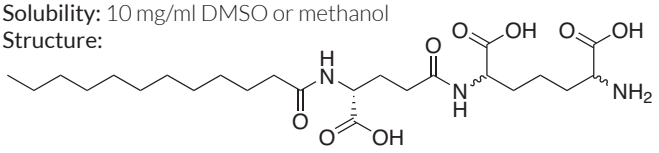
Synonym: Lauroyl-γ-D-glutamyl-meso-diaminopimelic acid

Formula: $C_{24}H_{43}N_3O_8$

Molecular weight: 501.61

Solubility: 10 mg/ml DMSO or methanol

Structure:



METHOD

Preparation of stock solution (1 mg/ml)

1. Add 100 µl DMSO (or methanol) to 1 mg of C12-iE-DAP.
2. Vortex until completely resuspended.
3. Once C12-iE-DAP is resuspended, add 900 µl endotoxin-freewater (provided).
4. Prepare aliquots and store at -20°C. Further dilutions can be prepared by adding the appropriate amount of endotoxin-free water.

NOD1 activation using C12-iE-DAP

C12-iE-DAP can be used to activate NOD1 in cells expressing this receptor such as HEK-Blue™ NOD1 cells. These cells were designed to study NOD1 stimulation by monitoring NF-κB activation. Stimulation of HEK-Blue™ NOD1 cells with a NOD1 agonist activates NF-κB, which induces the production of SEAP (secreted embryonic alkaline phosphatase). Levels of SEAP can be easily determined using HEK-Blue™ Detection, a cell culture medium that allows the detection of SEAP as it is secreted by the cells. For more information visit: https://www.invivogen.com/hek-blue-nod1.

1. Add 20 µl of C12-iE-DAP at various concentrations (10 ng-10 µg/ml) per well of a 96-well plate.
2. Prepare a cell suspension (~280,000 cells per ml) in HEK-Blue™ Detection medium and immediately add 180 µl of the cell suspension (~50,000 cells) to each C12-iE-DAP-containing well.
3. Incubate the plate for 6-24 h at 37°C, 5% CO_2.
4. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat.Code
HEK-Blue™ hNOD1 cells	Human NOD1 reporter cells	hkb-hnod1
HEK-Blue™ mNOD1 cells	Murine NOD1 reporter cells	hkb-mnod1
HEK-Blue™ Detection	SEAP detection medium	hb-det2
iE-DAP	NOD1 ligand	tlrl-dap
Tri-DAP	NOD1 ligand	tlrl-tdap

TECHNICAL SUPPORT
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