iE-DAP

PGN-like molecule - NOD1 ligand

Catalog # tlrl-dap

For research use only

Version # 07J17-SV

PRODUCT INFORMATION

Content:

- 5 mg γ-D-Glu-mDAP (iE-DAP)
- 1.5 ml sterile endotoxin-free water

Storage :

- iE-DAP is provided as a sterile white lyophilized powder and shipped at room temperature. Store at -20˚C.
- Upon resuspension, aliquote iE-DAP and store at -20˚C.
- Product is stable 1 year at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

DESCRIPTION

γ-D-Glu-mDAP (iE-DAP) is a dipeptide present in the peptidoglycan (PGN) of a subset of bacteria that include Gram-negative bacilli and particular Gram-positive bacteria such as *Bacillus subtilis* and *Listeria monocytogenes*[1]. iE-DAP is the minimal motif recognized by NOD1 (CARD4), an intracellular sensor expressed in multiple tissues including intestinal epithelia cells. Recognition of iE-DAP by NOD1 induces a signaling cascade involving the serine/threonine RIP2 (RICK, CARDIAK) kinase which interacts with IKK leading to the activation of NF-κB and the production of inflammatory cytokines such as TNFα and IL-6[2].

iE-DAP provided by InvivoGen is chemically synthesized and tested in HEK293 cells overexpressing NOD1.

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

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.

Synonym: γ-D-glutamyl-meso-diaminopimelic acid
Formula: $C_{12}H_{21}N_3O_7$
Molecular weight: 319.31
Endotoxin level: <0.125 EU/ml

METHODS

Preparation of sterile stock solution (10 mg/ml)

Stimulation of NOD1 can be achieved with 10 µg/ml iE-DAP.

- Add 500 µl endotoxin-free water (provided) and vortex until complete solubilisation.

Evaluation of iE-DAP stimulation

The ability of iE-DAP to stimulate NOD1 has been tested in HEK293 cells stably expressing human Nod1 gene and an NF-κB-inducible SEAP reporter gene.

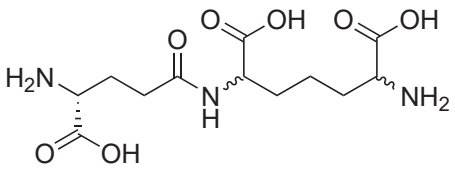
Note: HEK293 cells express endogenous levels of NOD1.

The amount of SEAP secreted in the supernatant is monitored by using HEK-Blue™ Detection medium, which turns blue in the presence of SEAP.

- 293/NOD1-SEAP cells were inoculated in HEK-Blue™ Detection medium (250,000 cells/ml) in a 96-well plate.
- Increasing concentrations of iE-DAP (1 to 100 µg/ml) were added per well.
- The plate was incubated overnight at 37°C, 5% CO_2.
- SEAP levels were assessed spectrophotometrically by reading the OD at 655 nm.

RELATED PRODUCTS

Product	Catalog Code
pUNO-hNOD1	puno-hnod1
pUNO-mNOD1	puno-mnod1
pNiFty-SEAP (Amp^R)	pnifty-seap
pNiFty2-SEAP (Zeo^R)	pnifty2-seap
HEK-Blue™ Detection	hb-det1



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