

# Tri-DAP

NOD1 ligand

Catalog # tlr1-tdap

For research use only

Version # 15A30-MM

## PRODUCT INFORMATION

### Contents:

• 1 mg L-Ala- $\gamma$ -D-Glu-mDAP (Tri-DAP). This product is chemically synthesized.

*Note:* Tri-DAP is a mixture of L-Ala- $\gamma$ -D-Glu-D-mDAP and L-Ala- $\gamma$ -D-Glu-L-mDAP.

• 1.5 ml endotoxin-free water

### Storage and stability:

- Tri-DAP is provided lyophilized and shipped at room temperature. Store at -20 °C.

- Upon resuspension, prepare aliquots of Tri-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 is validated using HEK-Blue™ NOD1 cells.

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

## DESCRIPTION

L-Ala- $\gamma$ -D-Glu-mDAP (Tri-DAP) is a tripeptide that consists of the dipeptide iE-DAP ( $\gamma$ -D-Glu-mDAP) and an L-Ala residue. Tri-DAP is 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*<sup>1</sup>. Tri-DAP is recognized by NOD1 (CARD4), an intracellular sensor expressed in multiple tissues, including intestinal epithelia cells. Recognition of Tri-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- $\kappa$ B and the production of inflammatory cytokines, such as TNF- $\alpha$  and IL-6<sup>2</sup>. Tri-DAP exhibits a ~3-fold higher ability to activate NF- $\kappa$ B than does iE-DAP<sup>3</sup>.

1. Chamailard 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. 3. Girardin SE. *et al.*, 2003. Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. *J Biol Chem.* 278(43):41702-8.

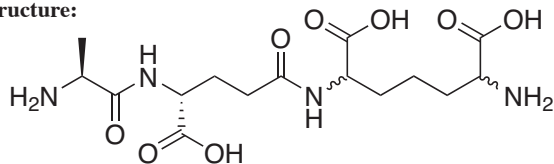
## CHEMICAL PROPERTIES

**Synonym:** L-alanyl- $\gamma$ -D-glutamyl-meso-diaminopimelic acid

**Formula:** C<sub>15</sub>H<sub>26</sub>N<sub>4</sub>O<sub>8</sub>

**Molecular weight:** 390.39

**Structure:**



## METHOD

### Preparation of stock solution (10 mg/ml)

- Add 100  $\mu$ l endotoxin-free water (provided) and vortex until completely dissolved.

- Prepare aliquots and store at -20 °C. Further dilutions can be prepared by adding the appropriate amount of endotoxin-free water.

### NOD1 activation using Tri-DAP

Tri-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- $\kappa$ B activation. Stimulation of HEK-Blue™ NOD1 cells with a NOD1 agonist activates NF- $\kappa$ B, 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: [www.invivogen.com/hek-blue-nod1](http://www.invivogen.com/hek-blue-nod1)

- Add 20  $\mu$ l of Tri-DAP at various concentrations (100 ng - 10  $\mu$ g/ml) per well of a 96-well plate.

- Prepare a cell suspension (~280,000 cells per ml) in HEK-Blue™ Detection medium and immediately add 180  $\mu$ l of the cell suspension (~50,000 cells) to each Tri-DAP-containing well.

- Incubate the plate for 6 - 24 h at 37 °C, 5% CO<sub>2</sub>.

- Determine SEAP levels using a spectrophotometer at 620 - 655 nm.

## RELATED PRODUCTS

Product	Catalog Code
<b>Reporter cells expressing NOD1</b>	
HEK-Blue™ hNOD1 cells (human NOD1)	hkb-hnod1
HEK-Blue™ mNOD1 cells (mouse NOD1)	hkb-mnod1
HEK-Blue™ Detection	hb-det2
<b>Other NOD1 agonists</b>	
C12-iE-DAP (acylated derivative of iE-DAP)	tlr1-c12dap
iE-DAP (synthetic triacylated lipoprotein)	tlr1-dap
NOD1 Test Strip (strips pre-coated with C12-iE-DAP)	tlrs-nod1
Tri-Lys (Tri-DAP negative control)	tlr1-tlys

## TECHNICAL SUPPORT

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