

iE-Lys

PGN-like molecule - iE-DAP Negative Control

Catalog # tlr1-lys

For research use only

Version # 07G13-MT

PRODUCT INFORMATION

Content:

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

Storage :

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

DESCRIPTION

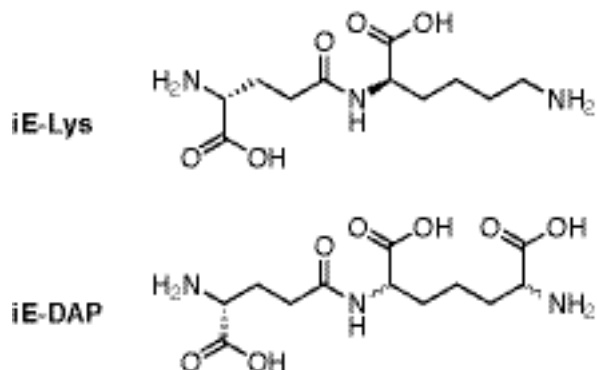
γ -D-Glu-Lys (iE-Lys) is a dipeptide present in the peptidoglycan (PGN) of Gram-positive bacteria. iE-Lys is not recognized by NOD1 (CARD4), in contrast to iE-DAP which is found mainly in Gram-negative bacteria. Thus iE-Lys can be used as a negative control for NOD1 activation studies with iE-DAP or its derivative C12-iE-DAP.

Synonym: γ -D-glutamyl-Lysine

Formula: $\text{C}_{11}\text{H}_{21}\text{N}_3\text{O}_5$

Molecular weight: 275.30

Endotoxin level: <0.125 EU/ml



METHODS

Preparation of sterile stock solution (10 mg/ml)

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

Evaluation of NOD1 activation

NOD1 activation can be studied in HEK293 cells stably expressing human Nod1 gene and an NF- κ B-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.

- Prepare a cell suspension of 293/NOD1-SEAP cells in HEK - Blue™ Detection medium at $\sim 250,000$ cells/ml.
- Add 20 μl of iE-DAP (NOD1 agonist) or iE-Lys (negative control) at increasing concentrations (1 to 100 $\mu\text{g/ml}$) in a well of 96-well plate.
- Add 180 μl of 293/NOD1-SEAP cell suspension (45,000 cells/well)
- Incubate the plate overnight at 37°C in 5% CO_2 .
- Assess SEAP levels spectrophotometrically by reading the OD at 655 nm.

RELATED PRODUCTS

Product	Catalog Code
pUNO1-hNOD1	puno1-hnod1
pUNO1-mNOD1	puno1-mnod1
pNiFty-SEAP (Amp ^R)	pnifty-seap
pNiFty2-SEAP (Zeo ^R)	pnifty2-seap
HEK-Blue™ Detection	hb-det2
iE-DAP	tlr1-dap
C12-IE-DAP	tlr1-c12dap

TECHNICAL SUPPORT

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