c-di-AMP
Cyclic diadenylate monophosphate, a CDS ligand
Catalog code: tlrl-nacda, tlrl-nacda-5
http://www.invivogen.com/cdiamp

For research use only. Not for use in humans.
Version 18E29-MM

PRODUCT INFORMATION
Contents
- c-di-AMP is provided lyophilized and is available in two sizes:
  - 1 mg c-di-AMP: tlrl-nacda
  - 5 mg (5 x 1 mg) c-di-AMP: tlrl-nacda-5
Note: c-di-AMP is sterile filtered prior to lyophilization.
* Storage and stability
  - c-di-AMP is shipped at room temperature and should be stored at -20°C.
  - Lyophilized product is stable for 1 year when properly stored.
  - Upon resuspension, aliquot of c-di-AMP and store at -20°C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.
Quality control
- Purity and structure has been determined by LC/MS and NMR ≥ 95%.
- The ability of c-di-AMP to induce type I interferon (IFN) has been confirmed in THP1-Blue™ ISG cells.
- The absence of bacterial contamination (e.g. lipoproteins & endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION
Bis-(3’-5’)-cyclic dimeric adenosine monophosphate (c-di-AMP) is a bacterial second messenger implicated in the control of cell wall metabolism, osmotic stress responses and sporulation. Detection of c-di-AMP by the host cytoplasmic surveillance pathway (CSP) is known to elicit type I interferon (IFN) responses through a signaling axis that involves STING, TBK1 and IRF3. Involvement of the helicase DDX41 in the recognition of c-di-AMP has been suggested. Research has also demonstrated that c-di-AMP exerts strong adjuvant activities when delivered by the mucosal route. CDS ligands, such as c-di-AMP, trigger type I IFN production and the induction of interferon stimulated genes (ISG) through interferon regulatory factors (IRFs). To facilitate their study, InvivoGen has developed stable reporter cells in two well established immune cell models, the human monocytic cell line THP-1 cell line and the murine RAW 264.7 macrophages. These cells express a reporter gene, either SEAP or luciferase, under the control of an IRF-inducible promoter.


CHEMICAL PROPERTIES
Synonym: c-di-AMP sodium salt
CAS number: 54447-84-6
Formula: C20H22N10O12P2 •2Na
Molecular weight: 702.38
Solubility: 50 mg/ml in water
Structure:

METHODS
Preparation of stock solution (1 mg/ml)
Stimulation of CDSs can be achieved with 1-100 μg/ml c-di-AMP.
- Add 1 ml of endotoxin-free water to 1 mg c-di-AMP to obtain a solution at 1 mg/ml.
- Mix the solution by pipetting up and down.

Induction of type I IFNs in THP1-Lucia™ ISG cells
Induction of type I IFNs with c-di-AMP can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express all the CDSs, with the exception of DAI. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:
- Resuspend c-di-AMP, as described above.
- Stimulate cells with 1-100 μg/ml c-di-AMP for 16-48 hours.
- Monitor induction of type I IFNs by assessing luciferase reporter gene expression using QUANTI-Luc™.
Note: Alternatively, THP1-Blue™ ISG cells, an IRF-SEAP reporter cell line, can be used.

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