

# c-di-AMP

## Cyclic diadenylate monophosphate, a CDS ligand

Catalog code: tlr1-nacda, tlr1-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: tlr1-nacda
  - 5 mg (5 x 1 mg) c-di-AMP: tlr1-nacda-5

*Note: c-di-AMP is sterile filtered prior to lyophilization.*

- endotoxin-free water; 1.5 ml with #tlr1-nacda and 10 ml with #tlr1-nacda-5

### 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, prepare aliquots 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<sup>1,2</sup>. Involvement of the helicase DDX41 in the recognition of c-di-AMP has been suggested<sup>3</sup>. Research has also demonstrated that c-di-AMP exerts strong adjuvant activities when delivered by the mucosal route<sup>4,5</sup>.

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 THP-1 cell line and the murine RAW 264.7 macrophages. These cells express a reporter gene, either SEAP or Lucia luciferase, under the control of an IRF-inducible promoter.

1. Jin L. *et al.*, 2011. MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. *J Immunol.* 187(5):2595-601. 2. Burdette DL. *et al.*, 2011. STING is a direct innate immune sensor of cyclic di-GMP. *Nature.* 478(7370):515-8. 3. Parvatiyar K. *et al.*, 2012. The helicase DDX41 recognizes the bacterial secondary messengers cyclic di-GMP and cyclic di-AMP to activate a type I interferon immune response. *Nat. Immunol.* 3(12):1155-61. 4. Ebensen T. *et al.*, 2011. Bis-(3',5')-cyclic dimeric adenosine monophosphate: strong Th1/Th2/Th17 promoting mucosal adjuvant. *Vaccine.* 29(32):5210-20. 5. Škrnjug I. *et al.*, 2014. The mucosal adjuvant cyclic di-AMP exerts immune stimulatory effects on dendritic cells and macrophages. *PLoS One.* 9(4):e95728. 6. Unterholzner L. *et al.*, 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(11):997-1004. 7. Zhang Z. *et al.*, 2011. The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. *Nat Immunol.* 12(10):959-65. 8. Arakawa R. *et al.*, 2010. Characterization of LRRFIP1. *Biochem Cell Biol.* 88(6):899-906. 9. Lippmann J. *et al.*, 2010. IFNβ responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). *Cell Microbiol.* 10(12):2579-88.

## CHEMICAL PROPERTIES

**Synonym:** c-di-AMP sodium salt

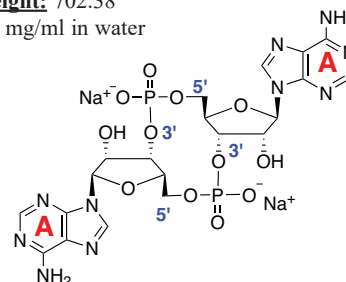
**CAS number:** 54447-84-6

**Formula:** C<sub>20</sub>H<sub>22</sub>N<sub>10</sub>O<sub>12</sub>P<sub>2</sub> • 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<sup>6,8</sup>, with the exception of DAI<sup>9</sup>. 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 Lucia luciferase reporter gene expression using QUANTI-Luc™.

*Note: Alternatively, THP1-Blue™ ISG cells, an IRF-SEAP reporter cell line, can be used.*

## RELATED PRODUCTS

Product	Catalog Code
LyoVec™	lyec-12
QUANTI-Luc™	rep-qlc1
RAW-Lucia™ ISG cells	rawl-isg
RAW-Lucia™ ISG-KO-STING cells	rawl-kostg
THP1-Blue™ ISG cells	thp-isg
THP1-Lucia™ ISG cells	thpl-isg
<b>Other CDS ligands</b>	
c-di-GMP	tlr1-nacdg
HSV-60c/LyoVec™	tlr1-hsv60c
ISD/LyoVec™	tlr1-isdc
VACV-70/LyoVec™	tlr1-vav70c

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