

2'3'-c-di-AMP

Cyclic adenosine monophosphate: a STING ligand

Catalog # ttrl-nacda23

For research use only. Not for use in humans.

Version # 16C21-MM

PRODUCT INFORMATION

Content

- 500 µg of lyophilized 2'3'-c-di-AMP

Note: 2'3'-c-di-AMP is sterile filtered prior to lyophilization.

- 1.5 ml endotoxin-free water

Storage and stability

- 2'3'-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 2'3'-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 2'3'-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

2'3'-c-di-AMP is a synthetic analog of the bacterial second messenger 3'3'-cyclic adenosine monophosphate (c-di-AMP) which differ only by one phosphodiester linkage. c-di-AMP is a potent immunostimulant in mammals. It induces production of type I interferons (IFNs) by directly binding to the endoplasmic reticulum-resident receptor STING (stimulator of interferon genes)¹. Interestingly, it has been reported that its analog 2'3'-c-di-AMP can induce modest antitumoral activity in animal models². When used at low concentrations in various cellular assays, 2'3'-c-di-AMP induces higher levels of type I IFNs than does c-di-AMP. STING ligands such as c-di-AMP induce type I IFNs and activate 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: THP-1 human monocytes and RAW 264.7 murine macrophages. These cells express a reporter gene (SEAP or Lucia luciferase), under 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. Corrales L *et al.*, 2015. Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. *Cell Rep.* 11(7):1018-30. 3. Unterholzner L *et al.*, 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(11):997-1004. 4. 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. 5. Arakawa R. *et al.*, 2010. Characterization of LRRFIP1. *Biochem Cell Biol.* 88(6):899-906. 6. Lippmann J. *et al.*, 2010. IFN-beta 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: 2'3'-c-di-AMP sodium salt; 2',5'-3',5'-c-diAMP; cyclic (adenosine-(2'→5')-monophosphate-adenosine-(3'→5')-monophosphate); c[A(2',5')pA(3',5')p]

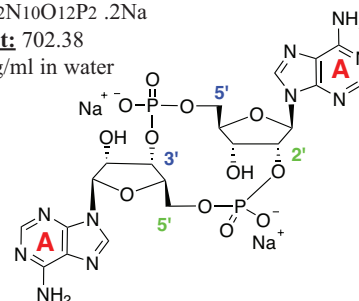
CAS number: 1638241-77-6

Formula: C₂₀H₂₂N₁₀O₁₂P₂ .2Na

Molecular weight: 702.38

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (1 mg/ml)

Stimulation of STING can be achieved with 1-100 µg/ml 2'3'-c-di-AMP.

- Add 1 ml of endotoxin-free water to 1 mg of 2'3'-c-di-AMP.

- Vortex until completely dissolved.

Induction of type I IFNs in THP1-Lucia ISG cells

Induction of type I IFNs with 2'3'-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^{3,5}, with the exception of DAI⁶. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:

- Resuspend 2'3'-c-di-AMP, as described above.

- Stimulate cells with 1-100 µg/ml 2'3'-c-di-AMP for 16-48 hours.

- Monitor induction of type I IFNs by measuring the levels of IRF-induced Lucia luciferase in the cell culture supernatant using QUANTI-Luc™, a Lucia luciferase detection reagent.

RELATED PRODUCTS

Product	Catalog Code
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
Other STING ligands	
2'3'-cGAMP	ttrl-nacga23
3'3'-cGAMP	ttrl-nacga
c-di-AMP	ttrl-nacda
c-di-GMP	ttrl-nacdg
DMXAA	ttrl-dmx

TECHNICAL SUPPORT

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