

c-di-AMP Control (5'-pApA)

Linear diadenylate monophosphate: a negative control for c-di-AMP

Catalog # tlr1-napapa

For research use only. Not for use in humans.

Version # 16C02-MM

PRODUCT INFORMATION

Content:

- 1 mg of lyophilized chemically synthesized c-di-AMP Control (5'-pApA)

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

- 1.5 ml endotoxin-free water

Storage and stability:

- c-di-AMP Control 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 Control 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: $\geq 95\%$

- The inability of c-di-AMP Control 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

c-di-AMP Control, also known as 5'-pApA, is a linear dinucleotide analog obtained after hydrolysis of cyclic diadenylate monophosphate (c-di-AMP) by phosphodiesterases¹. c-di-AMP is a cyclic dinucleotide (CDN) second messenger produced in bacteria. CDNs including c-di-AMP bind the cytosolic DNA sensor STING (stimulator of interferon genes) and induce the production of type I interferons (IFNs). Due to its linear conformation, 5'-pApA is intended to serve as a negative control for c-di-AMP in type I IFN induction assays. The importance of dinucleotide conformation has been well established in bacteria, where RNA regulatory riboswitches are able to discriminate between biologically active CDNs and their corresponding linear dinucleotides^{2,4}.

To facilitate the study of CDNs and the IFN pathway, InvivoGen has developed stable reporter cells in the human monocytic THP-1 cell line. These cells express a reporter gene (either SEAP or the secreted Lucia luciferase) under the control of an interferon regulatory factors (IRF)-inducible promoter. As expected, c-di-AMP Control does not induce a type I IFN response in this immune cell model.

1. Huynh TN. & Woodward JJ., 2016. Too much of a good thing: regulated depletion of c-di-AMP in the bacterial cytoplasm. *Curr Opin Microbiol.* 30:22-29. 2. Ren A. et al., 2015. Structural Basis for Molecular Discrimination by a 3',3'-cGAMP Sensing Riboswitch. *Cell Rep.* 11(1):1-12. 3. Gao J. et al., 2015. Identification and characterization of phosphodiesterases that specifically degrade 3'3'-cyclic GMP-AMP. *Cell Res.* 25(5):539-50. 4. Witte CE. et al., 2013. Cyclic di-AMP is critical for *Listeria monocytogenes* growth, cell wall homeostasis, and establishment of infection. *MBio.* 4(3):e00282-13. 5. Unterholzner L. et al., 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(11):997-1004. 6. 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. 7. Arakawa R. et al., 2010. Characterization of LRRFIP1. *Biochem Cell Biol.* 88(6):899-906. 8. Lippmann J. et al., 2010. IFN β 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

CAS number: 3593-47-3

Source: Synthetic

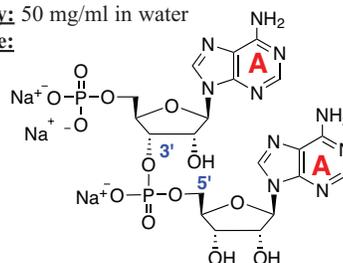
Synonym: 5'-pApA sodium salt

Formula: C₂₀H₂₃N₁₀O₁₃P₂ .3Na

Molecular weight: 742.38

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (1 mg/ml):

- Add 1 ml of endotoxin-free water to 1 mg of c-di-AMP Control to obtain a solution at 1 mg/ml.

- Mix the solution by pipetting.

c-di-AMP Control is intended for use as a negative control in type I IFN induction assays based on the activation of STING. Below is a standard protocol for determining type I IFN induction with a STING agonist such as c-di-AMP. Use c-di-AMP Control at the same concentration as c-di-AMP.

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 CDSS⁵⁻⁷, with the exception of DAI⁸. 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 and c-di-AMP Control, as described above.

- Stimulate cells with 1-100 μ g/ml c-di-AMP and c-di-AMP Control for 16-48 hours.

- Monitor the 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
c-di-AMP	tlr1-nacda
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

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