

c-di-GMP Control (5'-pGpG)

Linear diguanylate monophosphate: a negative control for c-di-GMP

Catalog # tlr1-napgg

For research use only. Not for use in humans.

Version # 16D11-MM

PRODUCT INFORMATION

Content:

- 1 mg of lyophilized c-di-GMP Control (5'-pGpG)

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

- 1.5 ml endotoxin-free water

Storage and stability:

- c-di-GMP 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-GMP 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: ≥ 95%

- The inability of c-di-GMP 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-GMP Control, also known as 5'-pGpG, is a linear dinucleotide analog obtained after hydrolysis of cyclic diguanylate monophosphate (c-di-GMP) by phosphodiesterases¹. c-di-GMP is a cyclic dinucleotide (CDN) second messenger produced in bacteria. CDNs including c-di-GMP 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'-pGpG is intended to serve as a negative control for c-di-GMP 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-GMP Control does not induce a type I IFN response in this immune cell model.

1. Shanahan CA. et al., 2016. Identification of c-di-GMP derivatives resistant to an EAL domain phosphodiesterase. *Biochemistry*. 52(2):365-77. 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. 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.

TECHNICAL SUPPORT

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CHEMICAL PROPERTIES

Synonym: 5'-pGpG sodium salt

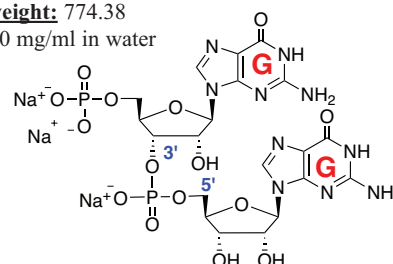
CAS number: 33008-99-0

Formula: C₂₀H₂₃N₁₀O₁₅P₂.3Na

Molecular weight: 774.38

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (1 mg/ml):

Stimulation of CDSs can be achieved with 10-100 µg/ml c-di-GMP Control.

- Add 1 ml of endotoxin-free water to 1 mg of c-di-GMP Control.

- 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-GMP Control 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-GMP Control, as described above.

- Stimulate cells with 10-100 µg/ml c-di-GMP Control 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
LyoVec™	lyec-12
QUANTI-Luc™	rep-qlc1
RAW-Lucia™ ISG cells	rawl-isg
RAW-Lucia™ ISG-KO-STING cells (STING knockout)	rawl-kostg
THP1-Blue™ ISG cells	thp-isg
THP1-Lucia™ ISG cells	thpl-isg
CDS ligands	
c-di-AMP	tlrl-nacda
c-di-GMP	tlrl-nacdg
HSV-60/LyoVec™	tlrl-hsv60c
VACV-70/LyoVec™	tlrl-vav70c