c-di-GMP

Cyclic diguanylate monophosphate, a CDS ligand

Catalog code: tlrl-nacdg, tlrl-nacdg-5

http://www.invivogen.com/cdigmp

For research use only. Not for use in humans.

Version 18E29-MM

PRODUCT INFORMATION

Contents

• c-di-GMP is provided lyophilized and is available in two sizes:
- 1 mg c-di-GMP: tlrl-nacdg
- 5 mg (5 x 1 mg) c-di-GMP: tlrl-nacdg-5

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

• endotoxin-free water; 1.5 ml with #tlrl-nacdg and 10 ml with #tlrl-nacdg-5

Storage and stability

- c-di-GMP 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 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-GMP 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

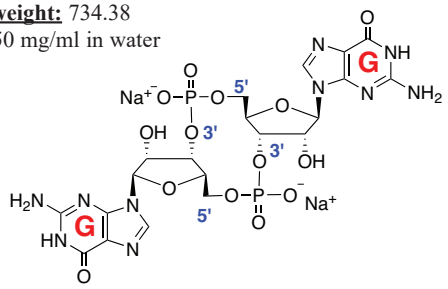
Bis-(3'-5')-cyclic dimeric guanosine monophosphate (c-di-GMP) is a second messenger produced in bacteria but not in mammals. This cyclic dinucleotide is involved in complex biological processes, such as biofilm formation, virulence and photosynthesis. Besides its role as an intracellular and intercellular signaling molecule in prokaryotes, c-di-GMP also affects eukaryotes. In mammals, c-di-GMP is recognized by STING, initiating a response characterized by the production of type I interferons through the TBK1/IRF3 axis[1, 2]. The helicase DDX41 may play a role in the recognition of c-di-GMP upstream of STING[3]. Studies have also demonstrated that c-di-GMP exhibits potent adjuvant properties[4].

CDS ligands, such as c-di-GMP, 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.Madhun AS. *et al.*, 2011.** Intranasal c-di-GMP-adjuvanted plant-derived H5 influenza vaccine induces multifunctional Th1 CD4+ cells and strong mucosal and systemic antibody responses in mice. Vaccine.29(31):4973-82. **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.** IFNbeta 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-GMP sodium salt
CAS number: 61093-23-0
Formula: $C_{20}H_{22}N_{10}O_{14}P_2 .2Na$
Molecular weight: 734.38
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.
- Add 1 ml of endotoxin-free water to 1 mg of c-di-GMP.
- 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 can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express all the CDSs[5-7], with the exception of DAI[8]. 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, as described above.
- Stimulate cells with 10-100 µg/ml c-di-GMP 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
Other CDS ligands	
c-di-AMP	tlrl-nacda
HSV-60/LyoVec™	tlrl-hsv60c
ISD/LyoVec™	tlrl-isdc
pCpGfree-giant/LyoVec™	tlrl-cpgfc
VACV-70/LyoVec™	tlrl-vav70c

TECHNICAL SUPPORT
InvivoGen USA (Toll-Free): 888-457-5873
InvivoGen USA (International): +1 (858) 457-5873
InvivoGen Europe: +33 (0) 5-62-71-69-39
InvivoGen Hong Kong: +852 3-622-34-80
E-mail: info@invivogen.com