3’3’-cGAMP

Cyclic [G(3’,5’)pA(3’,5’)p], a STING ligand

Catalog code: tlrl-nacga, tlrl-nacga-1, tlrl-nacga-2.5

https://www.invivogen.com/cgamp

For research use only. Not for use in humans.

Version 23A09-MM

PRODUCT INFORMATION

Contents

• 3’3’-cGAMP (formerly called cGAMP) is provided as a lyophilized powder and is available in three quantities:
- 500 µg 3’3’-cGAMP: tlrl-nacga
- 1 mg (2 x 500 µg) 3’3’-cGAMP: tlrl-nacga-1
- 2.5 mg (5 x 500 µg) 3’3’-cGAMP: tlrl-nacga-2.5

Note: 3’3’-cGAMP is sterile filtered prior to lyophilization.

• endotoxin-free water; 1.5 ml with #tlrl-nacga and tlrl-nacga-1 and 10 ml with #tlrl-nacga-2.5

Storage and stability

- Product is shipped at room temperature and should be stored at -20°C.
- Upon resuspension, prepare aliquots of 3’3’-cGAMP 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 3’3’-cGAMP to induce type I interferon (IFN) has been confirmed in cellular assays.
- The absence of bacterial contamination (endotoxins, peptidoglycans) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

3’3’-cGAMP (cyclic [G(3’,5’)pA(3’,5’)p], previously known as cGAMP) is a cyclic di-nucleotide produced by bacteria. 3’3’-cGAMP is also referred to as “canonical” cGAMP due the presence of the classical 3’-5’ phosphodiester linkages between the guanosine and the adenosine. Research has demonstrated that 3’3’-cGAMP binds to STING (stimulator of IFN genes) and subsequently induces TBK1-IRF3-dependent production of IFN-β[1]. Studies in bacteria revealed that 3’3’-cGAMP serves as a second messenger and plays a role in bacterial chemotaxis and colonization[2]. Structural and functional studies revealed that the canonical 3’3’-cGAMP produced by bacteria is distinct from the noncanonical 2’3’-cGAMP produced by mammalian cGAS[3]. Certain variants of STING are able to distinguish between canonical and noncanonical cGAMP[4]. 3’3’-cGAMP is more potent in activating IRF3 than c-di-IMP, c-di-AMP and c-diGMP, other 3’,5’ cyclic dinucleotides that also bind to STING[5].

To facilitate the study of cGAMP, InvivoGen provides stable reporter cells derived from 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 (secreted embryonic alkaline phosphatase) or the secreted Lucia luciferase, under the control of an IRF-inducible promoter.

1. Zhang X. et al., 2013. Cyclic GMP-AMP containing mixed phosphdiester linkages is an endogenous high-affinity ligand for STING. Mol Cell.51(2):226-35. **2. Davies B. et al., 2012.** Coordinated regulation of accessory genetic elements produces cyclic di-nucleotides for V. cholerae virulence. Cell. 149(2):358-70. **3. Gao P. et al., 2013.** Cyclic [G(2’,5’)pA(3’,5’)p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. Cell. 153(5):1094-107. **4. Diner E. et al., 2013.** The Innate Immune DNA Sensor cGAS Produces a Noncanonical Cyclic Dinucleotide that Activates Human STING. Cell Rep. 3(5):1355-61. **5. Burdette D. et al., 2011.** STING is a direct innate immune sensor of cyclic di-GMP. Nature. 478(7370):515-8.

CHEMICAL PROPERTIES

CAS number: 849214-04-6

Synonym: cyclic GMP-AMP; c-GpAp sodium salt

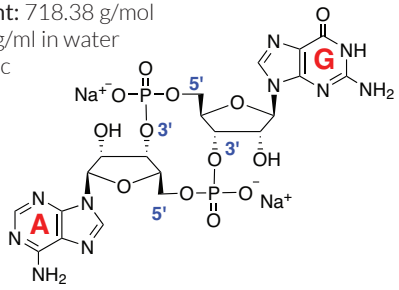
Formula: $C_{20}H_{22}N_{10}O_{13}P_2 \bullet 2Na$

Molecular weight: 718.38 g/mol

Solubility: 50 mg/ml in water

Source: Synthetic

Structure:



METHODS

Preparation of stock solution (1 mg/ml)

Stimulation of STING can be achieved with 0.1-100 µg/ml 3’3’-cGAMP.
- Add 500 µl endotoxin-free water (provided) to 500 µg 3’3’-cGAMP.
- Vortex until completely dissolved.

Induction of type I IFNs in THP1-Blue™ ISG cells

Induction of type I IFNs with 3’3’-cGAMP can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express STING and respond to 3’3’-cGAMP. A protocol for the induction of type I IFNs using THP1-Blue™ ISG cells, an IRF-SEAP reporter cell line, is given below:
- Resuspend 3’3’-cGAMP, as described above.
- Stimulate cells with 0.1-100 µg/ml 3’3’-cGAMP for 16-48 hours.
- Monitor induction of type I IFNs by measuring the levels of IRF-induced SEAP in the cell culture supernatant using QUANTI-Blue™ Solution, a SEAP detection reagent.

Note: Alternatively, THP1-Lucia™ ISG cells, an IRF-Lucia luciferase reporter cell line, can be used.

RELATED PRODUCTS

Product	Description	Cat. Code
2’3’-cGAMP	STING ligand	tlrl-nacga23
c-di-AMP	STING ligand	tlrl-nacda
c-di-GMP	STING ligand	tlrl-nacdg
RAW-Lucia™ ISG cells	Mouse Macrophages	rawl-isg
RAW-Lucia™ ISG-KO-STING cells	STING knockout cells	rawl-kostg
THP1-Blue™ ISG cells	Human monocytes	thp-isg
THP1-Lucia™ ISG cells	Human monocytes	thpl-isg

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 Asia: +852 3622-3480
E-mail: info@invivogen.com