

3'3'-cGAMP

Cyclic [G(3',5')pA(3',5')p], a STING ligand
Catalog code: tlr1-nacga, tlr1-nacga-1, tlr1-nacga-2.5

<http://www.invivogen.com/cgamp>

For research use only. Not for use in humans.

Version 18H01-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: tlr1-nacga
- 1 mg (2 x 500 µg) 3'3'-cGAMP: tlr1-nacga-1
- 2.5 mg (5 x 500 µg) 3'3'-cGAMP: tlr1-nacga-2.5

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

• endotoxin-free water; 1.5 ml with #tlr1-nacga and tlr1-nacga-1 and 10 ml with #tlr1-nacga-2.5

Storage and stability

- 3'3'-cGAMP 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 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 THP1-Blue™ ISG cells.
- 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-β¹. Studies in bacteria revealed that 3'3'-cGAMP serves as a second messenger and plays a role in bacterial chemotaxis and colonization². 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 cGAS³. Certain variants of STING are able to distinguish between canonical and noncanonical cGAMP⁴. 3'3'-cGAMP is more potent in activating IRF3 than c-di-IMP, c-di-AMP and c-di-GMP, other 3',5' cyclic dinucleotides that also bind to STING⁵.

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 phosphodiester 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

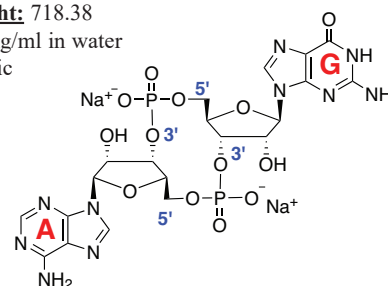
Formula: C₂₀H₂₂N₁₀O₁₃P₂ • 2Na

Molecular weight: 718.38

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™, a SEAP detection reagent.

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

RELATED PRODUCTS

Product	Catalog Code
2'2'-cGAMP	tlr1-nacga22
2'3'-cGAMP	tlr1-nacga23
c-di-AMP	tlr1-nacda
c-di-GMP	tlr1-nacd
RAW-Lucia™ ISG cells	rawl-isg
RAW-Lucia™ ISG-KO-STING cells	rawl-kostg
THP1-Blue™ ISG cells	thp-isg
THP1-Lucia™ ISG cells	thp1-isg

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