

2'3'-cGAMP

Cyclic [G(2',5')pA(3',5')p], a STING ligand

Catalog code: tlr1-nacga23-02, tlr1-nacga23, tlr1-nacga23-1, tlr1-nacga23-5

<http://www.invivogen.com/23-cgamp>

For research use only. Not for use in humans.

Version 18E22-MM

PRODUCT INFORMATION

Contents

• 2'3'-cGAMP is provided as a lyophilized powder and is available in four quantities:

- 200 µg 2'3'-cGAMP: tlr1-nacga23-02
- 500 µg 2'3'-cGAMP: tlr1-nacga23
- 1 mg (2 x 500 µg) 2'3'-cGAMP: tlr1-nacga23-1
- 5 mg (5 x 1 mg) 2'3'-cGAMP: tlr1-nacga23-5

Note: 2'3'-cGAMP is sterile filtered prior to lyophilization.

• 1.5 ml endotoxin-free water is provided with tlr1-nacga23-02, tlr1-nacga23 and tlr1-nacga23-1 and 10 ml endotoxin-free water is provided with tlr1-nacga23-5

Storage and stability

- 2'3'-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 2'3'-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 2'3'-cGAMP to induce type I interferon (IFN) has been confirmed in THP1-Blue™ ISG cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

2'3'-cGAMP (cyclic [G(2',5')pA(3',5')p]) is produced in mammalian cells by cGAS (cGAMP synthase) in response to double-stranded DNA in the cytoplasm. 2'3'-cGAMP is also referred to as “noncanonical” cGAMP due to the presence of the atypical 2'-5' phosphodiester linkage between the guanosine and the adenosine. Similar to the canonical 3'3'-cGAMP, 2'3'-cGAMP serves as a second messenger to activate innate immune responses by binding to STING (stimulator of IFN genes) and subsequently inducing the TBK1-IRF3-dependent production of IFN-β¹. Structural and functional studies revealed that noncanonical 2'3'-cGAMP is distinct from the canonical 3'3'-cGAMP produced by bacteria^{2,3}. 2'3'-cGAMP binds to STING with a much greater affinity than 3'3'-cGAMP¹. Interestingly, certain variants of STING are able to distinguish between noncanonical and canonical cGAMP².

To facilitate the study of STING, 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. 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. **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.

CHEMICAL PROPERTIES

CAS number: 1441190-66-4

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

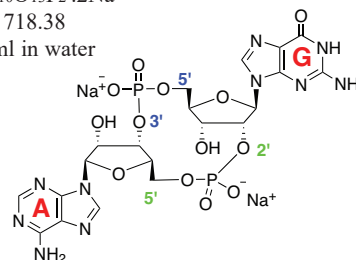
Formula: C₂₀H₂₂N₁₀O₁₃P₂ .2Na

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 2'3'-cGAMP.

- Resuspend 2'3'-cGAMP with endotoxin-free water (provided).
 - Add 200 µl endotoxin-free water to 200 µg 2'3'-cGAMP.
 - Add 500 µl endotoxin-free water to 500 µg 2'3'-cGAMP.
 - Add 1 ml endotoxin-free water to 1 mg 2'3'-cGAMP.
- Mix the solution by pipetting up and down.

IRF induction in THP1-Lucia ISG cells

Induction of type I IFNs with 2'3'-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 2'3'-cGAMP. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:

- Resuspend 2'3'-cGAMP, as described above.
- Stimulate cells with 0.1-100 µg/ml 2'3'-cGAMP for 16-48 hours.
- Monitor IRF induction by measuring Lucia luciferase in the cell culture supernatant using QUANTI-Luc™, a Lucia luciferase detection reagent.

Note: Alternatively, you can use THP1-Blue™ ISG cells, an IRF-SEAP reporter cell line, with QUANTI-Blue™.

RELATED PRODUCTS

Product	Catalog Code
3'3'-cGAMP	tlr1-nacga
c-di-AMP	tlr1-nacda
c-di-GMP	tlr1-nacdgd
RAW-Lucia™ ISG cells	rawl-isg
RAW-Lucia™ ISG-KO-STING cells (STING knockout)	rawl-kostgt
THP1-Lucia™ ISG cells	thp1-isg
THP1-Dual™ Cells	thpd-nfis
THP1-Dual KO-STING Cells	thpd-kostgt

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 3622-3480

E-mail: info@invivogen.com

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www.invivogen.com