

2'2'-cGAMP

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

Catalog # tlr1-nacga22, tlr1-nacga22-1

For research use only. Not for use in humans.

Version # 15L01-MM

PRODUCT INFORMATION

Content:

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

- 500 µg 2'2'-cGAMP: tlr1-nacga22
- 1 mg (2 x 500 µg) 2'2'-cGAMP: tlr1-nacga22-1

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

- 1.5 ml endotoxin-free water

Storage and stability:

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

DESCRIPTION

Cyclic guanosine monophosphate- adenosine monophosphate (cyclic GMP-AMP, cGAMP) represents the first cyclic di-nucleotide discovered in metazoa and functions as a second messenger that triggers type I interferon (IFN) production in response to cytosolic DNA¹. cGAMP has also been identified in the bacterium *Vibrio cholerae* and shown to play a role in bacterial chemotaxis and colonization. In mammals, cGAMP is synthesized by cGAMP synthetase (cGAS), a newly characterized cytosolic DNA sensor. cGAS binds DNA and catalyzes the synthesis of cGAMP from ATP and GTP. Subsequently, cGAMP binds the endoplasmic reticulum adaptor STING (stimulator of IFN genes) leading to the activation of IRF3 (IFN regulatory factor 3) and the induction of IFN-β.

To determine the structure of cGAMP produced in mammalian cells, structural and functional analysis of three synthetic cGAMP isomers was performed². 2'3'-cGAMP, cyclic [G(2',5')pA(3',5')p] was identified as the isomer formed by mammalian cGAS in presence of dsDNA. 2'2'-cGAMP, cyclic [G(2',5')pA(2',5')p] is most similar to 2'3'-cGAMP in terms of STING binding and type I IFN induction³. 3'3'-cGAMP, cyclic [G(3',5')pA(3',5')p] is the isomer produced by bacteria. Certain variants of STING are able to distinguish between cGAMP isomers⁴.

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.

CHEMICAL PROPERTIES

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

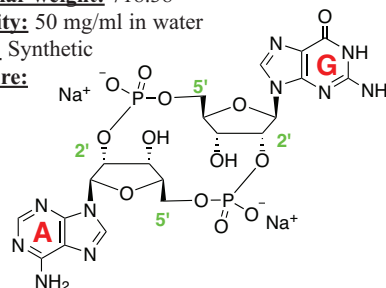
Formula: C₂₀H₂₂N₁₀O₁₃P₂ .2Na

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 2'2'-cGAMP.

- Add 500 µl endotoxin-free water (provided) to 500 µg 2'2'-cGAMP.
- Mix the solution by pipetting up and down.

Induction of type I IFNs in THP1-Lucia ISG cells

Induction of type I IFNs with 2'2'-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 2'2'-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 2'2'-cGAMP, as described above.
- Stimulate cells with 0.1-100 µg/ml 2'2'-cGAMP 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.

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

References

1. Wu J. *et al.*, 2013. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science* 339(6121):826-30.
2. 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.
3. 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.
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.

RELATED PRODUCTS

Product	Catalog Code
2'3'-cGAMP	tlr1-nacga23
3'3'-cGAMP	tlr1-nacga
THP1-Blue™ ISG cells	thp-isg
THP1-Lucia™ ISG cells	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 Hong Kong: +852 3-622-34-80

E-mail: info@invivogen.com