3’3’-cGAMP Control (pGpA)

Linear guanosine monophosphate- adenosine monophosphate; Negative control for 3’3’-cGAMP

Catalog # tlrl-napgpa

For research use only. Not for use in humans.

Version # 15K27-MM

PRODUCT INFORMATION

Content:

- 1 mg of lyophilized chemically synthesized 3’3’-cGAMP Control (pGpA)

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

- 1.5 ml endotoxin-free water

Storage and stability:

- 3’3’-cGAMP Control 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 Control 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 inability of 3’3’-cGAMP Control 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

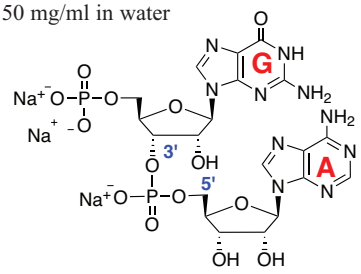
3’3’-cGAMP Control, also known as pGpA, is a linear dinucleotide analog obtained after hydrolysis of cyclic guanosine monophosphate- adenosine monophosphate (3’3’-cGAMP) by phosphodiesterases[1]. 3’3’-cGAMP, a second messenger molecule produced in bacteria, binds the cytosolic DNA sensor STING (stimulator of interferon genes) and induces the production of type I interferons (IFNs). Due to its linear conformation, pGpA is intended to serve as a negative control for 3’3’-cGAMP in type I IFN induction assays. The importance of dinucleotide conformation has been well established in bacteria, where RNA regulatory riboswitches are able to discriminate between biologically active CDNs and their corresponding linear dinucleotides[1-3].

To facilitate the study of cyclic dinucleotides (CDNs) and the IFN pathway, InvivoGen has developed stable reporter cells in the human monocytic THP-1 cell line. These cells express a reporter gene (either SEAP or the secreted Lucia luciferase) under the control of an interferon regulatory factors (IRF)-inducible promoter. As expected, 3’3’-cGAMP Control does not induce a type I IFN response in this immune cell model.

1. Gao J. et al., 2015. Identification and characterization of phosphodiesterases that specifically degrade 3'3'-cyclic GMP-AMP. Cell Res. 25(5):539-50. **2. Ren A. et al., 2015.** Structural Basis for Molecular Discrimination by a 3',3'-cGAMP Sensing Riboswitch. Cell Rep. 11(1):1-12. **3. Smith K. et al., 2012.** Structural and biochemical characterization of linear dinucleotide analogues bound to the c-di-GMP-I aptamer. Biochemistry. 51(1):425-32. **4. Unterholzner L. *et al.*, 2010.** IFI16 is an innate immune sensor for intracellular DNA.Nat Immunol. 11(11):997-1004. **5. 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. **6. Arakawa R. *et al.*, 2010.** Characterization of LRRFIP1. Biochem Cell Biol. 88(6):899-906. **7. 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

CAS number: 20137-01-3
Source: Synthetic
Synonym: 3’5’-pGpA sodium salt
Formula: $C_{20}H_{23}N_{10}O_{14}P_2 .3Na$
Molecular weight: 758.38
Solubility: 50 mg/ml in water
Structure:



METHODS

Preparation of stock solution (1 mg/ml):

- Add 1 ml of endotoxin-free water to 1 mg of 3’3’-cGAMP Control.
- Mix the solution by pipetting.

3’3’-cGAMP Control is intended for use a negative control in type I IFN induction assays based on the activation of stimulator of interferon genes (STING). Below is a standard protocol for determining type I IFN induction with a STING agonist such as 3’3’-cGAMP. Use 3’3’-cGAMP Control at the same concentration as 3’3’-cGAMP.

Induction of type I IFNs in THP1-Lucia 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 all the CDSs[4-6], with the exception of DAI[7]. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:

- Resuspend 3’3’-cGAMP and 3’3’-cGAMP Control, as described above.
- Stimulate cells with 0.1-100 µg/ml 3’3’-cGAMP and 3’3’-cGAMP Control for 16-48 hours.
- Monitor the 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.

RELATED PRODUCTS

Product	Catalog Code
3’3’-cGAMP	tlrl-nacga
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

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