# 3'3'-cGAMP Fluorinated

Fluorinated cyclic diadenylate monophosphate: a STING ligand

Catalog code: tlrl-nacgaf-05

https://www.invivogen.com/cgamp-fluorinated

For research use only Version 23L14-MM

### PRODUCT INFORMATION

#### Contents

- 5 x 100 μg of lyophilized 3'3'-cGAMP Fluorinated
<u>Note:</u> 3'3'-cGAMP Fluorinated is sterile filtered prior to lyophilization.
- 2 x 1.5 ml endotoxin-free water

#### Storage and stability:

- Product is shipped at room temperature and should be stored at -20°C. - Upon resuspension, prepare aliquots of 3'3'-cGAMP Fluorinated 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 have been determined by LC/MS and NMR: ≥ 95%

- The ability of 3'3'-cGAMP Fluorinated to induce type I interferon (IFN) has been confirmed using cellular assays.

- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue^ $\rm TLR2$  and HEK-Blue^ $\rm TLR4$  cells.

### DESCRIPTION

3'3'-cGAMP Fluorinated (c-[2'FdGMP]-[2'FdAMP]) is a synthetic analog of cyclic guanosine monophosphate- adenosine monophosphate (cyclic GMP-AMP, cGAMP). 3'3'-cGAMP is a cyclic di-nucleotide produced by bacteria. It is also referred to as «canonical» cGAMP due the presence of the classical 3'-5' phosphodiester linkages between the guanosine and the adenosine. It has been reported that cGAMP binds STING (stimulator of IFN genes) and subsequently induces TBK1-IRF3-dependent production of IFN- $\beta^1$ . 3'3'-cGAMP Fluorinated is composed of two 2'-deoxynucleosides with a fluorine atom at 2' position of each nucleoside.

The incorporation of fluorine into biologically active molecules is commonly used in medicinal chemistry to improve their metabolic stability or to modulate physicochemical properties such as lipophilicity<sup>2, 3</sup>. Moreover, the introduction of a fluorine atom can change the biological activities of a molecule. Interestingly, when used at low concentrations in various cellular assays, 3'3'-cGAMP Fluorinated induces higher levels of type I IFNs than does cGAMP.

STING ligands such as cGAMP induce type I IFNs and activate interferon stimulated genes (ISG) through IRFs. To facilitate their study, InvivoGen has developed stable reporter cells in two well established immune cell models: THP-1 human monocytes and RAW 264.7 murine macrophages. These cells express a reporter gene (SEAP or Lucia luciferase), under 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. Liu P. et al., 2008. Fluorinated Nucleosides: Synthesis and biological implication. J Fluor Chem. 129(9): 743-766. 4. Böhm HJ. et al., 2004. Fluorine in medicinal chemistry. Chembiochem. 5(5):637-43.

### CHEMICAL PROPERTIES

### Source: Synthetic

**Synonyms:** 2'Fluoro-cyclic dGMP-dAMP, c-(2'FdGMP)-(2'FdAMP) sodium salt

Formula: C20H20F2N10O11P2 •2Na

Molecular weight: 678.40 (free acid)



### **METHODS**

#### Preparation of stock solution (500 µg/ml):

Stimulation of STING can be achieved with 100 ng-10  $\mu g/ml$  3'3'-cGAMP Fluorinated.

1. Briefly centrifuge the vial before opening to dislodge any lyophilized material that may be dispersed on the wall or cap of the vial. Carefully open the vial lid to avoid any loss of product.

2. Add 200  $\mu l$  of endotoxin-free water to 100  $\mu g$  of 3'3'-cGAMP Fluorinated.

3. Vortex until completely dissolved.

#### Induction of type I IFNs in THP1-Lucia<sup>™</sup> ISG cells

Induction of type I IFNs with 3'3'-cGAMP Fluorinated can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express all the cytosolic DNA sensors, with the exception of DAI. A protocol for the induction of type I IFNs using THP1-Lucia<sup>~</sup> ISG cells, an IRF-luciferase reporter cell line, is given below:

1. Resuspend 3'3'-cGAMP Fluorinated, as described above.

Stimulate cells with 0.1-100 µg/ml 3'3'-cGAMP Fluorinated for 16-48 h.
Monitor induction of type I IFNs by measuring the levels of IRF-induced Lucia luciferase in the cell culture supernatant using QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia luciferase detection reagent.

### RELATED PRODUCTS

## Product Catalog Code

3'3'-cGAMP c-di-GMP c-di-GMP Fluorinated RAW-Lucia<sup>™</sup> (IRF-Lucia luciferase) ISG cells RAW-Lucia<sup>™</sup> ISG-KO-STING cells THP1-Dual<sup>™</sup> (NF-κB-SEAP & IRF-Luc) cells THP1-Dual<sup>™</sup> KI-hSTING-R232 cells tlrl-nacga tlrl-nacdg tlrl-nacdgf-05 rawl-isg rawl-kostg thpd-nfis thpd-r232



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