

c-di-AMP Fluorinated

Fluorinated cyclic diadenylate monophosphate: a STING ligand

Catalog code: tlr1-nacdaf-2

<https://www.invivogen.com/cdiamp-fluorinated>

For research use only. Not for use in humans.

Version 23A10-MM

PRODUCT INFORMATION

Contents

- 2 x 100 µg of lyophilized c-di-AMP Fluorinated

Note: c-di-AMP Fluorinated is sterile filtered prior to lyophilization.

- 2 x 1.5 ml endotoxin-free water

Storage and stability

- c-di-AMP Fluorinated is shipped at room temperature. Upon receipt, store at -20°C.

- Upon resuspension, prepare aliquots of c-di-AMP 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 has been determined by LC/MS and NMR: ≥ 95%
- The ability of c-di-AMP 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™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

c-di-AMP Fluorinated (c-di[2'F-dAMP]) is a synthetic analog of cyclic diadenylate monophosphate (c-di-AMP), a bacterial second messenger that is a potent immunostimulant in mammals. It induces production of type I interferons (IFNs) by directly binding to the endoplasmic reticulum-resident receptor STING (stimulator of interferon genes)^{1,2}. c-di-AMP Fluorinated is composed of two 2'-deoxyadenosines 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^{3,4}. 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, c-di-AMP Fluorinated induces higher levels of type I IFNs than does c-di-AMP.

STING ligands such as c-di-AMP induce type I IFNs and activate interferon stimulated genes (ISG) through interferon regulatory factors (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. Jin L. *et al.*, 2011. MPYS is required for IFN response factor 3 activation and type I IFN production in the response of cultured phagocytes to bacterial second messengers cyclic-di-AMP and cyclic-di-GMP. *J Immunol.* 187(5):2595-601. 2. Burdette DL. *et al.*, 2011. STING is a direct innate immune sensor of cyclic di-GMP. *Nature.* 478(7370):515-8. 3. 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. 5. Unterholzner L. *et al.*, 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(11):997-1004. 6. 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. 7. Arakawa R. *et al.*, 2010. Characterization of LRRFIP1. *Biochem Cell Biol.* 88(6):899-906. 8. Lippmann J. *et al.*, 2010. IFN beta 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

Source: Synthetic

Synonyms: 2'Fluoro-c-di-dAMP sodium salt, c-di(2'F-dAMP)

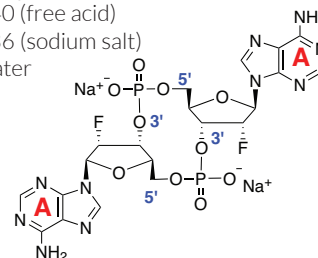
CAS number: 1427269-47-3

Formula: C₂₀H₂₀F₂N₁₀O₁₀P₂ • 2Na

Molecular weight: 662.40 (free acid)
706.36 (sodium salt)

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (100 µg/ml)

Stimulation of STING can be achieved with 300 ng -30 µg/ml c-di-AMP 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 1 ml of endotoxin-free water to 100 µg of c-di-AMP Fluorinated.
3. Vortex until completely dissolved.

Induction of type I IFNs in THP1-Lucia™ ISG cells

Induction of type I IFNs with c-di-AMP 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 sensors⁵⁻⁷, with the exception of DAI⁸. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:

1. Resuspend c-di-AMP Fluorinated, as described above.
2. Stimulate cells with 3-100 µg/ml c-di-AMP Fluorinated for 16-48 hours.
3. 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.

RELATED PRODUCTS

| Product | Catalog Code |
|---|---------------|
| c-di-AMP | tlr1-nacda |
| c-di-GMP | tlr1-nacd |
| c-di-GMP Fluorinated | tlr1-nacdgf-2 |
| RAW-Lucia™ (IRF-Lucia luciferase) ISG cells | rawl-isg |
| RAW-Lucia™ ISG-KO-STING cells | rawl-kostg |
| THP1-Dual™ (NF-κB-SEAP & IRF-Luc) cells | thpd-nfis |
| THP1-Dual™ KO-STING cells | thpl-kostg |
| THP1-Dual™ KI-hSTING-A162 cells | thpd-a162 |
| THP1-Dual™ KI-hSTING-R232 cells | 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

 **InvivoGen**
www.invivogen.com