

cAIMP Difluor

cAIMP difluorinated: a STING ligand

Catalog code: tlr1-nacaidf-05

<https://www.invivogen.com/caimp-difluor>

For research use only

Version 23L13-MM

PRODUCT INFORMATION

Contents

• 500 µg (2 x 250 µg) cAIMP Difluor provided lyophilized

Note: cAIMP Difluor is sterile filtered prior to lyophilization.

• 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 cAIMP Difluor 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 biological activity 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

cAIMP Difluor is a derivative of cAIMP, an analog of the bacterial cyclic dinucleotide (CDN) 3'3'-cGAMP¹. cAIMP and cAIMP Difluor are novel STING (stimulator of interferon genes)-activating synthetic CDNs. Unlike natural CDNs, whose constituent nucleosides are guanosine and/or adenine, cAIMP and its derivatives contain one adenine nucleoside and one inosine nucleoside. cAIMP Difluor 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 lipophilicity². Moreover, the introduction of a fluorine atom can change the biological activities of a molecule. Indeed cAIMP Difluor (referred to as compound 52 by Lioux *et al.*¹) more potently induces interferon regulatory factor (IRF) and NF-κB pathways in a STING-dependent manner when compared to STING agonists such as 2'3'-cGAMP and DMXAA³. Interestingly, cAIMP Difluor is more resistant than 2'3'-cGAMP and cAIMP to cleavage by certain nuclease and phosphodiesterase enzymes¹. STING ligands such as cAIMP Difluor induce production of type I interferons (IFNs) through IRFs and of proinflammatory cytokines through the NF-κB pathway. 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 inducible SEAP and/or Lucia luciferase reporter genes under the control of an IRF-inducible or NF-κB-inducible promoter.

1. Lioux T. *et al.*, 2016. Design, synthesis, and biological evaluation of novel cyclic adenosine-inosine monophosphate (cAIMP) analogs that activate stimulator of interferon genes (STING). *J Med Chem.* 59(22):10253-10267. 2. Böhm HJ. *et al.*, 2004. Fluorine in medicinal chemistry. *Chembiochem.* 5(5):637-43. 3. Unterholzner L. *et al.*, 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(11):997-1004. 4. 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. 5. 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: c-(2'FdAMP-2'FdIMP) sodium salt

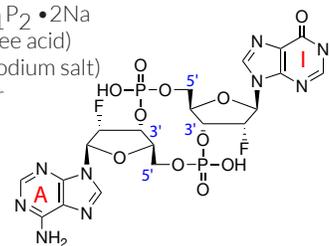
CAS number: 1951464-78-0

Formula: C₂₀H₂₁F₂N₉O₁₁P₂ • 2Na

Molecular weight: 663.4 (free acid)
707.4 (sodium salt)

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (1 mg/ml)

Stimulation of STING can be achieved with 100 ng -30 µg/ml cAIMP Difluor.

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 250 µl of endotoxin-free water to 250 µg of cAIMP Difluor.
3. Vortex until completely dissolved.

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

Induction of type I IFNs with cAIMP Difluor 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^{3,4}, with the exception of DAI⁵. A protocol for the induction of type I IFNs using THP1-Blue™ ISG cells, an IRF-luciferase reporter cell line, is given below:

1. Resuspend cAIMP Difluor as described above.
2. Stimulate cells with 100 ng-30 µg/ml cAIMP Difluor for 16-48 hours.
3. Monitor induction of type I IFNs by measuring the levels of IRF-induced SEAP (secreted embryonic alkaline phosphatase) in the cell culture supernatant using QUANTI-Blue™ Solution, a SEAP detection reagent.

RELATED PRODUCTS

Product	Catalog Code
STING ligands	
2'3'-cGAMP	tlrl-nacga23
cAIMP	tlrl-nacai
DMXAA	tlrl-dmx
STING reporter cells	
RAW-Blue™ (IRF-SEAP) ISG cells	raw-isg
THP1-Blue™ (IRF-SEAP) ISG cells	thp-isg
THP1-Dual™ (NF-κB-SEAP & IRF-Luc) cells	thpd-nfis
THP1-Dual™ KI-hSTING-R232 cells	thpd-r232

TECHNICAL SUPPORT

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