

# 2'3'-c-di-GMP

## Cyclic diguanylate monophosphate: a STING ligand

Catalog # ttrl-nacd23

For research use only. Not for use in humans.

Version # 17B02-MM

### PRODUCT INFORMATION

#### Content

- 500 µg of lyophilized 2'3'-c-di-GMP

*Note: 2'3'-c-di-GMP is sterile filtered prior to lyophilization.*

- 1.5 ml endotoxin-free water

#### Storage and stability

- 2'3'-c-di-GMP 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 2'3'-c-di-GMP 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 2'3'-c-di-GMP 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

2'3'-c-di-GMP is a synthetic analog of the bacterial second messenger 3'3'-cyclic diguanylate monophosphate (c-di-GMP) which differ only by one phosphodiester linkage. c-di-GMP 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)<sup>1,2</sup>. Interestingly, it has been reported that its analog 2'3'-c-di-GMP can induce modest antitumoral activity in animal models<sup>3</sup>. When used at low concentrations in various cellular assays, 2'3'-c-di-GMP induces higher levels of type I IFNs than does c-di-GMP.

STING ligands such as c-di-GMP 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. Corrales L *et al.*, 2015. Direct activation of STING in the tumor microenvironment leads to potent and systemic tumor regression and immunity. *Cell Rep.* 11(7):1018-30. 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. 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

**Synonym:** 2'3'-c-di-GMP sodium salt; 2',5'-3',5'-c-diGMP; cyclic (guanosine-(2'→5')-monophosphate-guanosine-(3'→5')-monophosphate); c[G(2',5')pG(3',5')p]

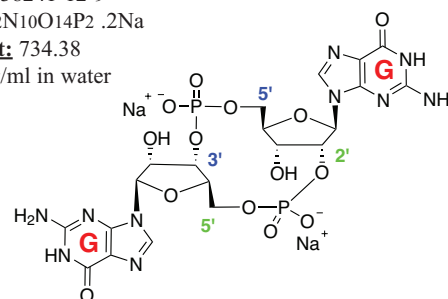
**CAS number:** 1638241-12-9

**Formula:** C<sub>20</sub>H<sub>22</sub>N<sub>10</sub>O<sub>14</sub>P<sub>2</sub> .2Na

**Molecular weight:** 734.38

**Solubility:** 50 mg/ml in water

**Structure:**



### METHODS

#### Preparation of stock solution (1 mg/ml)

Stimulation of STING can be achieved with 1-100 µg/ml 2'3'-c-di-GMP.

- Add 1 ml of endotoxin-free water to 1 mg of 2'3'-c-di-GMP.

- Vortex until completely dissolved.

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

Induction of type I IFNs with 2'3'-c-di-GMP 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<sup>4,6</sup>, with the exception of DAI<sup>7</sup>. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:

- Resuspend 2'3'-c-di-GMP, as described above.

- Stimulate cells with 1-100 µg/ml 2'3'-c-di-GMP 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.

### RELATED PRODUCTS

Product	Catalog Code
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
<b>Other STING ligands</b>	
2'3'-c-di-AMP	ttrl-nacda23
2'3'-c-di-AM(PS)2 (Rp,Rp)	ttrl-nacda2r-01
c-di-GMP	ttrl-nacd23
c-di-GMP Fluorinated	ttrl-nacd23f
VACV-70/LyoVec™	ttrl-vav70c

### TECHNICAL SUPPORT

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