

# 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp)

Bisphosphorothioate analog of 2'3'-cGAMP; a STING ligand

Catalog code: tlrl-nacga2srs-05

<https://www.invivogen.com/23-cGAMPS2-RS>

For research use only

Version 23L14-MM

## PRODUCT INFORMATION

### Contents

- 2 x 250 µg 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp) provided lyophilized

### Notes:

- This product is sterile filtered prior to lyophilization.
- 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp) is a mixture of Rp/Sp diastereoisomers.
- 1.5 ml endotoxin-free water

### Storage and stability

- 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp) is shipped at room temperature and should be stored at -20 °C.
- Upon resuspension, prepare aliquots of 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp) 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'-cGAM(PS)<sub>2</sub> (Rp/Sp) 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

2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp) is composed of Rp/Sp-isomers of the bisphosphorothioate analog of the mammalian cyclic dinucleotide (CDN) 2'3'-cGAMP<sup>1</sup>. CDNs are microbial messengers that induce innate immune responses in mammals by binding and activating STING (stimulator of interferon genes; also known as ERIS, MITA, MPYS, NET23 and TMEM173) leading to TBK1-IRF3-dependent type I interferon (IFN) production<sup>2,3</sup>. The most potent natural STING agonist in humans is 2'3'-cGAMP, a CDN produced in mammals by cGAS (cGAMP synthase) in response to double-stranded DNA in the cytoplasm<sup>4,5</sup>.

As STING agonists, 2'3'-cGAMP and other CDNs are being studied for their potential in immunotherapy and vaccination. An obstacle to their therapeutic utility is their ability to enzymatic hydrolysis by various nucleases and phosphodiesterases, some of which are highly specific. For example, the enzyme ENPP1 (ecto-nucleotide pyrophosphatase/phosphodiesterase) cleaves 2'3'-cGAMP but not other CDNs, such as its bisphosphorothioate analog, 2'3'-cGAM(PS)<sub>2</sub>. In fact, this analog is not only more stable than 2'3'-cGAMP but is also more potent<sup>1</sup>.

1. Li L. et al., 2014. Hydrolysis of 2'3'-cGAMP by ENPP1 and design of nonhydrolyzable analogs. *Nat Chem Biol.* 10(12):1043-8.
2. Wu J. et al., 2013. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. *Science* 339(6121):826-30.
3. Sun L. et al., 2013. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the Type I interferon pathway. *Science* 339(6121):786-91.
4. Gao P. et al., 2013. Cyclic [G(2',5')pA(3',5')p] is the metazoan second messenger produced by DNA-activated cyclic GMP-AMP synthase. *Cell.* 153(5):1094-107.
5. Ablasser A. et al., 2013. cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. *Nature.* 498(7454):380-4.

### TECHNICAL SUPPORT

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## CHEMICAL PROPERTIES

CAS number: 1637675-05-8 (free acid)

Synonyms: Di-thiophosphate analog of 2'3'-cGAMP; c-(RpSp)-di-ApsGps; 2'3'-cG'A'MP

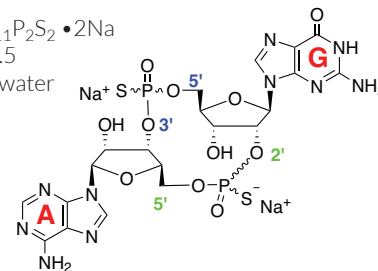
Formula: C<sub>20</sub>H<sub>22</sub>N<sub>10</sub>O<sub>11</sub>P<sub>2</sub>S<sub>2</sub> • 2Na

Molecular weight: 750.5

Solubility: 50 mg/ml in water

Source: Synthetic

Structure:



## METHODS

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

Stimulation of STING can be achieved with 0.1-100 µg/ml of 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp).

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 endotoxin-free water (provided) to 250 µg of 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp).
3. Vortex until completely dissolved.

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

To facilitate the study of cGAMP and its analog, InvivoGen provides stable reporter cells derived from two well established immune cell models, the human monocytic THP-1 cell line and the murine RAW 264.7 macrophages. These cells express a reporter gene, either SEAP (secreted embryonic alkaline phosphatase) or the secreted Lucia luciferase, under the control of an IRF-inducible promoter. A protocol for the induction of type I IFNs using THP1-Blue™ ISG cells, a SEAP reporter cell line, is given below:

1. Resuspend 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp), as described above.
2. Stimulate cells with 0.1-100 µg/ml 2'3'-cGAM(PS)<sub>2</sub> (Rp/Sp) for 16-48h.
3. Monitor induction of type I IFNs by measuring the levels of IRF-induced SEAP in the cell culture supernatant using QUANTI-Blue™ Solution, a SEAP detection reagent.

*Note: Alternatively, you can use THP1-Lucia™ ISG cells, an IRF-Lucia luciferase reporter cell line, with QUANTI-Luc™ 4 Lucia/Gaussia.*

## RELATED PRODUCTS

Product	Catalog Code
2'3'-cGAMP	tlrl-nacga23
QUANTI-Blue™ Solution	rep-qbs
RAW-Lucia™ ISG cells	raw-lisg
RAW-Lucia™ ISG-KO-STING cells	rawl-kostg
THP1-Blue™ ISG cells	thp-isg