2'3'-c-di-AM(PS)2 (Rp,Rp)

Bisphosphorothioate analog of c-di-AMP, Rp isomers, ADU-S100

Catalog code: tlrl-nacda2r-05, tlrl-nacda2r-1

https://www.invivogen.com/23-cdiAMPS2-RR

For research use only. Not for use in humans.

Version 24A02-MM

PRODUCT INFORMATION

Contents

• 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) is provided as a lyophilized powder and is available in two quantities:
- **500 µg (5 x 100 µg) 2'3'-c-di-AM(PS)$_2$ (Rp,Rp)** (#tlrl-nacda2r-05)
- **1 mg (2 x 500 µg) 2'3'-c-di-AM(PS)$_2$ (Rp,Rp)** (#tlrl-nacda2r-1)

Note: 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) is sterile filtered prior to lyophilization.

• 1.5 ml endotoxin-free water

Storage and stability

- 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) is shipped at room temperature and should be stored at -20°C.
- Upon resuspension, prepare aliquots of 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) and store at -20 °C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- Purity (≥ 95%) and structure has been determined by LC/MS and NMR.
- Activation of STING 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'-c-di-AM(PS)$_2$ (Rp,Rp) is a Rp,Rp-isomer of the 2'3' bis-phosphorothioate analog of 3'3'-cyclic adenosine monophosphate (c-di-AMP). It is structurally identical to ADU-S100/MIW815, a clinically-relevant molecule developed by Aduro in collaboration with Novartis currently in clinical trials for the treatment of various cancers.

c-di-AMP is second messenger molecule produced by bacteria that has potent immunostimulant activity[1]. This cyclic dinucleotide (CDN) induces the production of type I interferons (IFNs) following its recognition by the endoplasmic reticulum-resident receptor STING (stimulator of interferon genes) and the recruitment of TBK1 (TANK-binding kinase 1) and IRF3 (interferon regulatory factor 3)[2]. Notably, 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) has a higher affinity for STING than c-di-AMP due to the presence of a 2'-5', 3'-5' mixed linkage, as found in endogenous human CDNs produced by cGAS (cyclic GMP-AMP (cGAMP) synthase)[3]. It activates all known human STING alleles as well as murine STING. In addition, this analog contains two phosphorothioate diester linkages to protect it against degradation by phosphodiesterases that are present in host cells or in the systemic circulation[4]. The Rp, Rp dithio diastereoisomer has been found to induce higher type I IFN production compared to the Rp/Sp dithio diastereoisomers or c-di-AMP[3].

1. Woodward J.J. *et al.*, 2010. c-di-AMP secreted by intracellular Listeria monocytogenes activates a host type I interferon response. Science.328(5986):1703-5. 2. 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. 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. Yan H. *et al.*, 2008. Synthesis and immunostimulatory properties of the phosphorothioate analogues of cdiGMP. Bioorg. Med. Chem. Lett. 18, 5631–5634.

CHEMICAL PROPERTIES

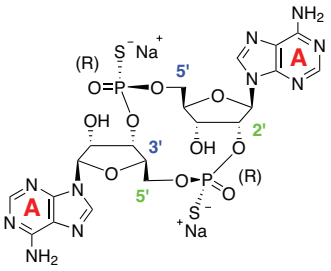
CAS number: 1638750-95-4 (sodium salt)

Synonyms:
- ADU-S100
- MIW815
- (R,R)-(2',3')c-diAM(PS)2, (2',3')-Rp,Rp-c-diAMPSS,

Formula: $C_{20}H_{22}N_{10}O_{10}P_2S_2 \bullet 2Na$

Molecular weight: 734.50 g/mol

Solubility: 50 mg/ml in water



METHODS

Preparation of stock solution

• Before opening the vial, centrifuge briefly and open the lid carefully to avoid any loss of product.
- Add 200 µl of endotoxin-free water to 100 µg of 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) to obtain a stock solution at 500 µg/ml, or
- Add 500 µl of endotoxin-free water to 500 µg of 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) to obtain a stock solution at 1 mg/ml.

• Vortex until completely dissolved.

Working concentration: 0.1 - 10 µg/ml

Activation of STING in THP1-Dual™ cells

Below is a protocol for monitoring the activation of STING by 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) using InvivoGen's THP1-Dual™ cells. These cells allow the simultaneous study of the NF-κB pathway, by monitoring the activity of SEAP, and the IRF (interferon regulatory factor) pathway, by assessing the activity of the secreted Lucia luciferase.
For more information, please visit https://www.invivogen.com/thp1-dual.

1. Add 20 µl of 2'3'-c-di-AM(PS)$_2$ (Rp,Rp) (10X final concentration) per well of a flat-bottom 96-well plate.
2. Add 20 µl of a positive control (e.g. 2'3'-cGAMP) to another well.
3. Prepare a suspension of THP1-Dual™ cells (~500,000 cells per ml) as detailed in the cell line data sheet.
4. Add 180 µl of cell suspension (~100,000 cells) per well.
5. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 hours.
6. Prepare QUANTI-Luc™ 4 Lucia/Gaussia (IRF assessment) and/or QUANTI-Blue™ Solution (NF-κB assessment) and carry out the measurements following the instructions on the data sheet.

RELATED PRODUCTS

Product	Catalog Code
THP1-Dual™ cells	thpd-nfis
THP1-Dual™ KO-STING cells	thpd-kostg
2'3'-cGAMP	tlrl-nacga23
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1

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