c-di-UMP

Cyclic di-uridine monophosphate; Negative Control for STING ligands

Catalog # tlrl-nacdu

For research use only. Not for use in humans.

Version # 15L02-MM

PRODUCT INFORMATION

Content:

- 1 mg of lyophilized c-di-UMP

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

- 1.5 ml endotoxin-free water

Storage and stability:

- c-di-UMP is shipped at room temperature and should be stored at -20°C. Lyophilized product is stable 1 year when properly stored.
- Upon resuspension, prepare aliquots of c-di-UMP and store at -20°C. Resuspended product is stable 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 inability of c-di-UMP 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

Cyclic di-uridine monophosphate (c-di-UMP) is a synthetic cyclic dinucleotide containing a pyrimidine base. Unlike the purine-containing cyclic dinucleotides (c-di-AMP, c-di-GMP and c-di-IMP; see Related Products), c-di-UMP is not predicted to bind strongly to STING[1]. The smaller size of the pyrimidine base is expected to prevent the formation of stacking interactions that are critical for proper binding to STING. Indeed, STING-expressing cells, such as THP1-Blue™ ISG cells, do not respond to c-di-UMP, even at high concentrations.

BACKGROUND

The innate immune system provides the first line of defense against infectious pathogens. Innate immune detection of intracellular DNA derived from viruses and invasive bacteria is important to initiate an effective protective response. This crucial step depends on cytosolic DNA sensors, which upon activation trigger the production of type I interferons (IFNs). Cytosolic DNA-mediated production of type I IFNs requires the transcription factor IRF3 (IFN regulatory factor 3), TBK1 (TANK-binding-kinase-1) and the transmembrane protein STING (stimulator of IFN genes)[2, 3].

STING ligands trigger type I IFN production and the induction of 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, the human monocytic THP-1 cell line and the murine RAW 264.7 macrophages.

1. Yin Q. *et al.*, 2012. Cyclic di-GMP sensing via the innate immune signaling protein STING. Mol Cell. 46(6):735-45. **2. Ishikawa H. & Barber GN., 2008.** STING is an endoplasmic reticulum adaptor that facilitates innate immune signalling. Nature. 455(7213):674-8. **3. Ishikawa H. *et al.*, 2009.** STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 461(7265):788-92. **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: c-di-UMP sodium salt

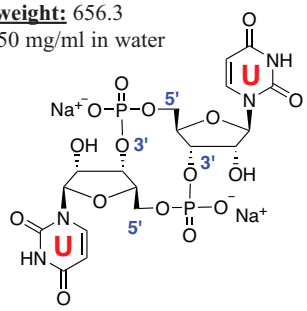
CAS number: 73120-97-5

Formula: $C_{18}H_{20}N_4O_{16}P_2 \cdot 2Na$

Molecular weight: 656.3

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (1 mg/ml)

- Add 1 ml of endotoxin-free water to the 1 mg c-di-UMP vial.
- Mix the solution by pipetting up and down.

Induction of type I IFNs in THP1-Blue ISG cells

Induction of type I IFNs with cyclic dinucleotides 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[4-6], with the exception of DAI[7]. A protocol for the induction of type I IFNs using THP1-Blue™ ISG cells, an IRF-secreted embryonic alkaline phosphatase (SEAP) reporter cell line, is given below:

- Resuspend cyclic dinucleotides, as described above.
- Treat cells with 1-100 µg/ml cyclic dinucleotides (see Related Products) and c-di-UMP (negative control) for 18-24 hours.
- Monitor induction of type I IFNs by measuring the levels of IRF-induced SEAP in the cell culture supernatant using QUANTI-Blue™, a SEAP detection reagent.

RELATED PRODUCTS

Product	Catalog Code
QUANTI-Blue™	rep-qb1
RAW-Blue™ ISG cells	raw-isg
RAW-Lucia™ ISG cells	rawl-isg
RAW-Lucia™ ISG-KO-STING cells (STING knockout)	rawl-kostg
THP1-Blue™ ISG cells	thp-isg
THP1-Blue™ ISG-KD STING cells (STING knockdown)	thp-kdstg
STING ligands - Cyclic dinucleotides	
c-di-AMP	tlrl-nacda
c-di-GMP	tlrl-nacdg
c-di-IMP	tlrl-nacdi
cGAMP	tlrl-nacga

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