c-di-IMP

Cyclic di-inosine monophosphate: a STING ligand

Catalog # tlrl-nacdi

For research use only. Not for use in humans.

Version # 15L02-MM

PRODUCT INFORMATION

Content:

- 1 mg of lyophilized c-di-IMP

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

- 1.5 ml endotoxin-free water

Storage and stability:

- c-di-IMP 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 c-di-IMP 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 c-di-IMP 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

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).

Cyclic di-inosine monophosphate (c-di-IMP) is a synthetic analog of the bacterial second messengers c-di-AMP and c-di-GMP. Although chemically different, c-di-IMP exhibits similar conformational features and biological properties as c-di-GMP. *In vitro* studies have revealed that c-di-IMP displays comparable capacities to c-di-GMP in promoting the activation and maturation of antigen presenting cells[1]. Furthermore, c-di-IMP has been shown to possess strong adjuvant properties when co-administered with an antigen by the mucosal route[1]. The immunostimulatory activity of c-di-IMP depends on the signaling molecule STING[2]. Cells with reduced STING expression respond weakly to c-di-IMP in contrast to cells expressing high levels of STING.

STING ligands, such as c-di-IMP, 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. Libanova R. *et al.*, 2010. The member of the cyclic di-nucleotide family bis-(3', 5')-cyclic dimeric inosine monophosphate exerts potent activity as mucosal adjuvant. Vaccine. 28(10):2249-58. **2. Burdette DL. *et al.*, 2011.** STING is a direct innate immune sensor of cyclic di-GMP. Nature. 478(7370):515-8. 5. AMP to activate a type I interferon immune response. Nat. Immunol. 3(12):1155-61. **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. Arakawa R. *et al.*, 2010.** Characterization of LRRFIP1. Biochem Cell Biol. 88(6):899-906. **6. 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-IMP sodium salt

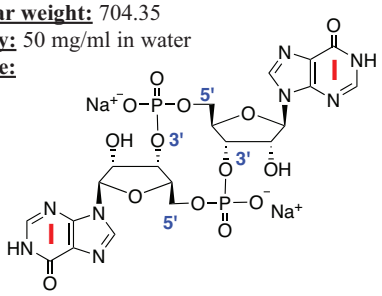
CAS number: 79940-41-3

Formula: $C_{20}H_{20}N_8O_{14}P_2 \cdot 2Na$

Molecular weight: 704.35

Solubility: 50 mg/ml in water

Structure:



METHODS

Preparation of stock solution (1 mg/ml)

Stimulation of cytosolic DNA sensors (CDSs) and STING can be achieved with 10-100 µg/ml c-di-IMP.

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

Induction of type I IFNs in THP1-Blue ISG cells

Induction of type I IFNs with c-di-IMP can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express all the CDSs[3-5], with the exception of DAI[6]. 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 c-di-IMP, as described above.
- Stimulate cells with 10-100 µg/ml c-di-IMP 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
Other STING ligands	
c-di-AMP	tlrl-nacda
c-di-GMP	tlrl-nacdg
c-di-UMP (Negative Control for STING ligands)	tlrl-nacdu
cGAMP	tlrl-nacga

TECHNICAL SUPPORT
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