Poly(dG:dC) naked

Synthetic double-stranded DNA; a CDS ligand

Catalog code: tlrl-pgcn

https://www.invivogen.com/poly-dgdc

For research use only

Version 20D07-MM

PRODUCT INFORMATION

Contents

- 200 µg Poly(dG:dC)
- 1.5 ml sterile endotoxin-free physiological water (150 mM NaCl)

Storage and stabiliy

- Poly(dG:dC) is provided lyophilized and shipped at room temperature. Store lyophilized product at -20°C. Lyophilized product is stable for 12 months when properly stored.
- Upon resuspension, prepare aliquots and store at -20°C. Resuspended product is stable for 12 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- The ability of intracellular Poly(dG:dC) to induce type I interferon (IFN) has been verified 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

Poly(deoxyguanylic-deoxycytidylic) acid (Poly(dG:dC)) is a synthetic repetitive double-stranded DNA (dsDNA). Intracellular poly(dG:dC) is detected by several cytosolic DNA sensors (CDS), such as cGAS[1], DAI[2], DDX41[2,3], IFI16[2,3] and LRRFIP1[2,4], triggering the production of type I interferons (IFNs). This induction of IFN appears to be mediated by the endoplasmic reticulum protein STING[1,3,5].

Moreover, Poly(dG:dC) activates the cytosolic DNA sensor AIM2 (absent in melanoma 2) to trigger inflammasome formation, leading to the secretion of the pro-inflammatory cytokines IL-1β and IL-18[6].

1. Unterholzner L., 2013. The interferon response to intracellular DNA: why so many receptors? Immunobiology. 218(11):1312-21. **2. Takaoka A. *et al.*, 2007.** DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. Nature. 448(7152):501-5. **3. 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. **4. Yang P. *et al.*, 2010.** The cytosolic nucleic acid sensor LRRFIP1 mediates the production of type I interferon via a beta-catenin-dependent pathway. Nat Immunol. 11(6):487-94. **5. 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. **6. Jones JW. *et al.*, 2010.** Absent in melanoma 2 is required for innate immune recognition of Francisella. PNAS, 107(21):9771-6.

CHEMICAL PROPERTIES

CAS number: 90385-88-9
Solubility: Physiological water (2 mg/ml)
Synonym: Poly(deoxyguanylic-deoxycytidylic) acid sodium salt
Working concentrations: 10 ng/ml to 1 µg/ml

METHODS

Preparation of stock solution (1 mg/ml)

- Add 200 µl of sterile endotoxin-free physiological water (provided) to 200 µg of poly(dG:dC).
- Vortex until completely dissolved.

Preparation of poly(dG:dC)/cationic lipid complex

To facilitate its intracellular delivery, poly(dG:dC) should be complexed with a cationic lipid transfection agent, such as LyoVec™. A protocol for the preparation of a poly(dG:dC)/LyoVec™ complex is given below:
-Rehydrate poly(dG:dC) as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring poly(dG:dC) and LyoVec™ to room temperature before use.
- In a sterile 1.5 ml microfuge tube, mix 1 µg of poly(dG:dC) with 100 µl of LyoVec™. Homogenize gently.
- Incubate at room temperature for 15 minutes to allow the formation of the complex.

Induction of type I IFNs

Induction of type I IFNs with poly(dG:dC) can be studied in a variety of cells including the monocytic cell line THP1-Blue™ ISG cells. These cells derive from the human THP-1 monocyte cell line by stable integration of an IFN regulatory factor (IRF)-inducible secreted embryonic alkaline phosphatase (SEAP) reporter construct. Levels of SEAP can be easily determined using a SEAP detection medium, such as QUANTI-Blue™ Solution.
For more information: https://www.invivogen.com/thp1-blue-isg.

1. Prepare poly(dG:dC)/cationic lipid complex as described above.
2. Dispense 20 µl of the poly(dG:dC)/cationic lipid complex (final concentration 10 ng/ml to 1 µg/ml) per well of a 96-well plate.
3. Prepare a cell suspension of THP1-Blue™ ISG cells according to the data sheet and add 180 µl of the cell suspension per well.
4. Incubate the plate for 6-24 h at 37°C, 5% CO_2.
5. Add 20 µl of supernatant per well into a flat-bottom 96-well plate.
6. Dispense 180 µl of QUANTI-Blue™ Solution per well.
7. Incubate the plate at 37°C for 1-3 h.
8. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Catalog Code
Poly(dA:dT)/LyoVec™	tlrl-patc
Poly(dA:dT) naked	tlrl-patn
Poly(dG:dC)/LyoVec™	tlrl-pgcc
LyoVec™	lyec-1
QUANTI-Blue™ Solution	rep-qbs
THP1-Blue™ ISG cells	thp-isg

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