Poly(dG:dC)/LyoVec™

Synthetic double-stranded DNA complexed with LyoVec[™]

Catalog code: tlrl-pgcc https://www.invivogen.com/poly-dgdc

For research use only

Version 20D07-MM

PRODUCT INFORMATION

Contents

- $4 \times 25 \,\mu g$ lyophilized poly(dG:dC)/LyoVec[™] Note: Each vial contains 25 μg of poly(dG-dC)•poly(dC-dG) complexed with $50 \,\mu g$ LyoVec.
- 10 ml sterile endotoxin-free water

Storage and stability

- Poly(dG:dC)/LyoVec[™] 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, store poly(dG:dC)/LyoVec[™] at 4 °C. Resuspended product is stable for 1 week when stored at 4 °C.

Quality control

- The ability of Poly(dG:dC)/LyoVec[™] 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 $^{\rm m}$ TLR2 and HEK-Blue $^{\rm m}$ TLR4 cells.

DESCRIPTION

Poly(deoxyguanylic-deoxycytidylic) acid (Poly(dG:dC)) is a synthetic repetitive double-stranded DNA (dsDNA). Poly(dG:dC) sodium salt is complexed with the cationic lipid LyoVec $^{^{\text{\tiny M}}}$ to facilitate its uptake.

Intracellular poly(dG:dC) is detected by several cytosolic DNA sensors (CDS), such as cGAS¹, DAI², DDX41². ³, IFI16². ³ and LRRFIP1².⁴, triggering the production of type I interferons (IFNs). This induction of IFN appears to be mediated by the endoplasmic reticulum protein STING¹.³.⁵. Moreover, Poly(dG:dC) activates the cytosolic DNA sensor AIM2 (absent in melanoma 2) to trigger inflammasome formation, leading to the secretion of the proinflammatory cytokines IL-1 β and IL-18⁶.

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.

METHODS

Preparation of stock solution (50 µg/ml)

- Add 500 μl sterile endotoxin-free water (provided) per vial of poly(dG:dC)/LyoVec". Mix gently. Allow at least 15 minutes for complete solubilization.

Working concentrations: 10 ng/ml to 5 µg/m

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 $^{\rm TM}$ 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. For more information: https://www.invivogen.com/thp1-blue-isg.

- 1. Dispense 20 µl of the poly(dG:dC)/cationic lipid complex (final concentration 10 ng/ml to 5 µg/ml) per well of a 96-well plate.
- 2. Prepare a cell suspension of THP1-Blue $^{\!\top\!}$ ISG cells according to the data sheet and add 180 μl of the cell suspension per well.
- 3. Incubate the plate for 6-24 h at 37°C, 5% CO₂.
- 4. Add 20 µl of supernatant per well into a flat-bottom 96-well plate.
- 5. Dispense 180 µl of QUANTI-Blue™ Solution per well.
- 6. Incubate the plate at 37°C for 1-3 h.
- 7. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

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



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