Poly(dA:dT)/LyoVec™

Double-stranded B DNA complexed with LyoVec™

Catalog code: tlrl-patc

https://www.invivogen.com/poly-dadt-lyovec

For research use only

Version 19D09-MM

PRODUCT INFORMATION

Contents

• 4 x 25 µg lyophilized poly(dA:dT)/LyoVec™

Note: Each vial contains 25 µg of poly(dA-dT)•poly(dT-dA) *complexed with 50 µg LyoVec.*

• 10 ml sterile endotoxin-free water

Storage and stability

- Poly(dA:dT)/LyoVec™ is provided lyophilized and shipped at room temperature. Store lyophilized product at -20 °C for up to 12 months.
- Upon resuspension, store poly(dA:dT)/LyoVec™ at 4 °C. Resuspended product is stable for 1 week when properly stored.

Quality control

- The ability of Poly(dA:dT)/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™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Poly(deoxyadenylic-deoxythymidylic) acid (Poly(dA:dT)) is a repetitive double-stranded DNA (dsDNA) sequence of poly(dA-dT)•poly(dT-dA). Poly(dA:dT) sodium salt, a synthetic analog of B-DNA, is complexed with the cationic lipid LyoVec™ to facilitate its uptake.

Intracellular poly(dA:dT) is detected by several cytosolic DNA sensors (CDS), such as cGAS, DAI, DDX41, IFI16 and LRRFIP1, triggering the production of type I interferons (IFNs)[1-3]. This induction of IFN appears to be mediated by the endoplasmic reticulum protein STING[1-3]. Moreover, poly(dA:dT) is recognized by AIM2 triggering the formation of an inflammasome and the subsequent secretion of IL-1β and IL-18[4]. Furthermore, transfected poly(dA:dT) can be transcribed by RNA polymerase III into dsRNA with a 5'-triphosphate moiety (5'ppp-dsRNA) which is a ligand for RIG-I[5,6]. Thus poly(dA:dT) is indirectly sensed by RIG-I leading to type I IFN production through the adaptor molecule IPS-1 and the TBK1/IRF3 pathway[7].

1. Unterholzner L., 2013. The interferon response to intracellular DNA: why so many receptors? Immunobiology. 218(11):1312-21. **2. 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. **3. 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. **4. Jones JW. *et al.*, 2010.** Absent in melanoma 2 is required for innate immune recognition of Francisella tularensis. PNAS, 107(21):9771-6. **5. Ablasser A. *et al.*, 2009.** RIG-I-dependent sensing of poly(dA:dT) through the induction of an RNA polymerase III-transcribed RNA intermediate. Nat Immunol. 10(10):1065-72. **6. Chiu YH. *et al.*, 2009.** RNA polymerase III detects cytosolic DNA and induces type I interferons through the RIG-I pathway. Cell. 138(3):576-91. **7. Takeshita F. & Ishii KJ., 2008.** Intracellular DNA sensors in immunity. Curr Opin Immunol. 20(4):383-8.

METHODS

Preparation of stock solution (50 µg/ml)

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

Induction of type I IFNs

1. Stimulate A549-Dual™ cells with 10ng/ml to 10 µg/ml of poly(dA:dT)/LyoVec™ for 18-24 hours.
2. Monitor induction of type I IFNs by measuring the levels of Lucia luciferase in the cell culture supernatants using QUANTI-Luc™, a Lucia luciferase detection reagent.

Induction of IL-1β in THP-1 cells

THP-1 cells are grown in RPMI 1640 medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine and antibacterial antibiotics such as penicillin/streptomycin or Normocin™. THP-1 cells are grown in suspension to a density of $1.0x10^6$ cells/ml in tissue culture flasks.

1. Treat THP-1 cells with 0.5 µM (300 ng/ml) PMA for 3 hours at 37 °C in 5% CO2.

Note: PMA treatment increases the phagocytic properties of these cells and induces the production of pro-IL-1β.

2. Wash cells gently with PBS and add fresh culture medium.
3. After 1 to 3 days, wash cells with PBS and add fresh culture medium.
4. Add 1 to 5 µg/ml poly(dA:dT)/LyoVec™.
5. Incubate from 6 hours to overnight at 37 °C in 5% CO2.

Note: The production of pro-IL-1β can be further increased by priming PMA-activated THP-1 cells with LPS.

6. The next day, detect mature IL-1β in the supernatant of poly(dA:dT)-activated THP-1 cells by Western blot, ELISA or using HEK-Blue™ IL-1β cells. Theses cells are specifically engineered to detect bioactive IL-1β.

For a more detailed protocol, see the technical data sheet HEK-Blue™ IL-1β cells, which is available on our website https://www.invivogen.com/hek-blue-il1b

RELATED PRODUCTS

Product	Catalog Code
A549-Dual™ cells	a549d-nfis
Poly(dG:dC)/LyoVec™	tlrl-pgcc
HEK-Blue™ IL-1β	hkb-il1b
QUANTI-Luc™	rep-qlc1

TECHNICAL SUPPORT
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