

# Poly(dA:dT) naked

Double-stranded B-DNA

Catalog code: tlrl-patn, tlrl-patn-1

<https://www.invivogen.com/poly-dadt-naked>

For research use only

Version 19D09-MM

## PRODUCT INFORMATION

### Contents

- Poly(dA:dT) is available in two quantities:
  - tlrl-patn: 200 µg
  - tlrl-patn-1: 1 mg
- 1.5 ml sterile endotoxin-free physiological water (150 mM NaCl)

### Storage and stability

- Poly(dA:dT) is provided lyophilized and shipped at room temperature. Store lyophilized product at -20 °C for up to 12 months.
- Upon resuspension, prepare aliquots of poly(dA:dT) 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(dA:dT) 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). It is a synthetic analog of B-DNA. 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)<sup>1-3</sup>. This induction of IFN appears to be mediated by the endoplasmic reticulum protein STING<sup>1-3</sup>. Moreover, poly(dA:dT) is recognized by AIM2 triggering the formation of an inflammasome and the subsequent secretion of IL-1β and IL-18<sup>4</sup>.

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<sup>5,6</sup>. 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<sup>7</sup>.

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.

## CHEMICAL PROPERTIES

CAS number: 86828-69-5

Synonym: Poly(deoxyadenylic-deoxythymidylic) sodium salt

Solubility: Physiological water (2 mg/ml)

## METHODS

### Preparation of stock solution (1 mg/ml)

1. Add 200 µl sterile endotoxin-free physiological water (provided) per vial of poly(dA:dT) to the 200 µg vial or 1 ml to the 1 mg vial.
2. Vortex until completely dissolved.

### Preparation of poly(dA:dT)/cationic lipid complex

In order to facilitate the intracellular delivery of poly(dA:dT), poly(dA:dT) should be complexed with a cationic lipid transfection agent, such as LyoVec™ (see Related Products on the next page). A protocol for the preparation of a poly(dA:dT)/LyoVec™ complex is given below:

1. Rehydrate poly(dA:dT) as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring poly(dA:dT) and LyoVec™ to room temperature before use.
2. In a sterile 1.5 ml microfuge tube, mix 1 µg poly(dA:dT) with 100 µl of LyoVec™. Homogenize gently.
3. 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(dA:dT) can be studied in a variety of cells including A549-Dual™ cells, a reporter cell line derived from the human A549 lung carcinoma cell line. These cells express an IFN regulatory factor (IRF)-inducible Lucia luciferase and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter genes. Stimulation of this cell line with poly(dA:dT)/LyoVec™ activates the IFN pathway inducing Lucia luciferase production.

1. Prepare poly(dA:dT)/cationic lipid complex as described above.
2. Stimulate A549-Dual™ cells with 10 ng/ml to 1 µg/ml of poly(dA:dT)/cationic lipid complex for 18-24 hours.
3. 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.

## TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

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### Induction of IL-1 $\beta$ 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<sup>6</sup> cells/ml in tissue culture flasks.

1. Treat THP-1 cells with 0.5  $\mu$ M (300 ng/ml) PMA for 3 hours at 37 °C in 5% CO<sub>2</sub>.

*Note: PMA treatment increases the phagocytic properties of these cells and induces the production of pro-IL-1 $\beta$ .*

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  $\mu$ g/ml poly(dA:dT)/cationic lipid complex.

5. Incubate from 6 hours to overnight at 37 °C in 5% CO<sub>2</sub>.

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

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

For a more detailed protocol, see the technical data sheet HEK-Blue™ IL-1 $\beta$  cells, which is available on our website

<https://www.invivogen.com/hek-blue-il1b>

## RELATED PRODUCTS

Product	Catalog Code
A549-Dual™ cells	a549d-nfis
5'ppp-dsRNA	tlrl-3prna
5'ppp-dsRNA Control	tlrl-3prnac
HEK-Blue™ IL-1 $\beta$	hkb-il1b
LyoVec™	lyec-1
Normocin™	ant-nr1
Poly(dA:dT)/LyoVec™	tlrl-patc
Poly(dG:dC)/LyoVec™	tlrl-pgcc
Poly(dG:dC) naked	tlrl-pgcn
QUANTI-Luc™	rep-qlc1

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