Poly(dA:dT) Rhodamine

Rhodamine labeled double-stranded B-DNA

Catalog # tlrl-patrh

For research use only

Version # 16F27-MM

PRODUCT INFORMATION

Content:

• 10 µg Poly(dA:dT) Rhodamine
• 1.5 ml endotoxin-free water

Storage:

- Poly(dA:dT) Rhodamine is provided lyophilized and shipped at room temperature. Store lyophilized product at -20 °C for up to 12 months.
- Upon resuspension, prepare aliquots and store poly(dA:dT) Rhodamine at -20 °C. Resuspended product is stable for 12 months when properly stored. Avoid repeated freeze-thaw cycles. Protect from light.

DESCRIPTION

Poly(dA:dT) Rhodamine was chemically labeled by covalent coupling of a rhodamine probe containing a reactive alkylating group. This confers fluorescent properties to Poly(dA:dT) with a slight reduction of pattern recognition receptor (PRR) stimulatory activity.

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) 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)[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.

CHEMICAL PROPERTIES

CAS number: 86828-69-5
Synonym: Poly(deoxyadenylic-deoxythymidylic) sodium salt
Solubility: Water (1 mg/ml)

Spectral properties of Rhodamine

Excitation λ max: 546 nm
Emission λ max: 576 nm

APPLICATIONS

Poly(dA:dT) Rhodamine can be used for various applications:
- flow cytometry
- fluorescent and confocal microscopy.

METHODS

Preparation of stock solution (100 µg/ml)

- Add 100 µl endotoxin-free water (provided) per vial of 10 µg poly(dA:dT) Rhodamine. Mix by pipetting up and down.

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

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

-Rehydrate poly(dA:dT) Rhodamine as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring poly(dA:dT) Rhodamine and LyoVec™ to room temperature before use.
- In a sterile 1.5 ml microfuge tube, mix 1 µg poly(dA:dT) Rhodamine with 100 µl of LyoVec™. Homogenize gently.
- Incubate at room temperature for 15 minutes to allow the formation of the complex.

Fluorescent *in vitro* labeling with Poly(dA:dT) Rhodamine

The following protocol describes a method to detect poly(dA:dT) Rhodamine in murine macrophages RAW-Blue™ cells.
- Prepare a RAW-Blue™ cell suspension (500,000 cells/ml) in DMEM, 4.5 g/L glucose, and 10% (v/v) heat-inactivated fetal bovine serum.
- Add 180 µl of the RAW-Blue™ cell suspension per well of a 96-well plate.
- Stimulate cells with 100 ng-10 µg/ml poly(dA:dT) Rhodamine for 16h.
- Rinse cells with PBS in order to remove free poly(dA:dT) Rhodamine.
- Analyze fluorescent labeling using flow cytometry, fluorescent and confocal microscopy.

TECHNICAL SUPPORT
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Induction of type I IFNs

Induction of type I IFNs with poly(dA:dT) Rhodamine can be studied in a variety of cells including immortalized murine embryonic fibroblasts (MEFs), the murine B16 melanoma cell line and HEK293 cells.

- Prepare poly(dA:dT) Rhodamine/cationic lipid complex.
- Stimulate cells with 30 ng/ml to 10 µg/ml poly(dA:dT) Rhodamine/cationic lipid complex for 18-24 hours.
- Monitor induction of type I IFNs by measuring the levels of IFN-α and/or IFN-ß produced in the cell culture supernatants by ELISA or by using InvivoGen's SEAP reporter cells. InvivoGen provides HEK-Blue™ IFN-α/β cells that detect human type I IFNs, and B16-Blue™ IFN-α/β cells which detect murine type I IFNs.

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.

- Treat THP-1 cells with 0.5 µM (300 ng/ml) PMA for 3 hours at 37 °C in 5% CO_2.

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

- Wash cells gently with PBS and add fresh culture medium.
- After 1 to 3 days, wash cells with PBS and add fresh culture medium.
- Add 1 to 5 µg/ml poly(dA:dT) Rhodamine/cationic lipid complex.
- Incubate from 6 hours to overnight at 37 °C in 5% CO_2.

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

-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-1R cells. Theses cells are specifically engineered to detect bioactive IL-1β.

RELATED PRODUCTS

Product	Catalog Code
B16-Blue™ IFNα/β	bb-ifnab
HEK-Blue™ IFNα/β	hkb-ifnab
HEK-Blue™ IL-1R	hkb-il1r
LyoVec™	lyec-1
Normocin™	ant-nr1
Poly(dA:dT)/LyoVec™	tlrl-patc
Poly(dA:dT) naked	tlrl-patn
Poly(dG:dC)/LyoVec™	tlrl-pgcc
Poly(dG:dC) naked	tlrl-pgcn
5'ppp-dsRNA	tlrl-3prna
5'ppp-dsRNA Control	tlrl-3prnac
RAW-Blue™	raw-sp