# Poly(I:C) HMW Rhodamine

## High Molecular Weight

Rhodamine labeled synthetic analog of dsRNA; TLR3 ligand

Catalog code: tlrl-picr

https://www.invivogen.com/polyic-rhodamine

For research use only

Version 21A27-MM

## PRODUCT INFORMATION

**Contents**

- 10 µg Poly(I:C) HMW Rhodamine
- 1.5 ml sterile endotoxin-free water

**Storage and stability**

- Poly(I:C) HMW Rhodamine is shipped at room temperature. Lyophilized product can be stored at -20°C for up to 6 months. Protect from light.
- Upon resuspension, Poly(I:C) HMW Rhodamine should be aliquoted and stored at -20°C. Protect from light. Resuspended product is stable for 3 months at -20°C. Avoid repeated freeze-thaw cycles.

**Quality control**

- Human TLR3 (hTLR3) activity tested using HEK-Blue™ hTLR3 cells
- Rhodamine fluorescence evaluated on RAW-Blue™ cells using FACS analysis.

**Spectral properties of Rhodamine**

**Excitation λ max:** 546 nm

**Emission λ max:** 576 nm

## DESCRIPTION

Poly(I:C) HMW Rhodamine was chemically labeled by covalent coupling of a rhodamine probe containing a reactive alkylating group. This confers fluorescent properties to poly(I:C) HMW with a slight reduction of TLR3 recognition (see figure 2 on the next page).

Polyinosinic-polycytidylic acid (poly(I:C)) is a synthetic analog of double stranded RNA (dsRNA), a molecular pattern associated with viral infection. Both natural and synthetic dsRNAs are known to induce type I interferons (IFN) and other cytokines production. Poly(I:C) is recognized by Toll-like receptor 3 (TLR3)[1, 2]. Upon poly(I:C) recognition, TLR3 activates the transcription factor interferon regulatory factor 3 (IRF3), through the adapter protein Toll-IL-1 receptor (TIR) domain-containing adapter inducing IFN-β (TRIF, also known as TICAM-1)[3]. Activation of IRF3 leads to the production of type I IFNs, especially IFN-β. A second pathway involves the recruitment of TNF receptor-associated factor 6 (TRAF6) or receptor interacting protein 1 (RIP1), with the subsequent activation of the transcription factors NF-κB and AP-1[4]. Activation of this pathway triggers the production of inflammatory cytokines and chemokines such as TNF-α, IL-6 and CXCL10. Poly(I:C) is also recognized by the cytosolic RNA helicases retinoic acid-inducible protein I (RIG-I) and melanoma differentiation-associate gene 5 (MDA-5)[5].

**1. Alexopoulou L. *et al.*, 2001.** Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature, 413:732-8. **2. Matsumoto M. *et al.*, 2002.** Establishment of a monoclonal antibody against human Toll-like receptor 3 that blocks double-stranded RNA-mediated signaling. BBRC 293:1364-9. **3. Yamamoto M. *et al.*, 2003.** Cutting edge: A novel Toll/Il-1 receptor domain-containing adapter that preferentially activates the IFN-β promoter in the Toll-like receptor signaling. Science 301:640. **4. Kawai T. & Akira S., 2008.** Toll-like receptor and RIG-I-like receptor signaling. Ann N Y Acad Sci. 1143:1-20. **5. Kato H. *et al.*, 2006.** Small interfering RNAs mediate sequence-independent gene suppression and induce immune activation by signaling through toll-like receptor 3. Nature 441:101-5.

## APPLICATIONS

Poly(I:C) Rhodamine can be used for various applications:

- flow cytometry
- fluorescent and confocal microscopy.

## METHODS

**Preparation of sterile stock solution (100 µg/ml)**

Stimulation of TLR3 can be achieved with 10 ng-10 µg/ml Poly(I:C) Rhodamine.

1. Add 100 µl of the endotoxin-free water provided to the 10 µg Poly(I:C) HMW Rhodamine vial to obtain a solution at 100 µg/ml.
2. Homogenize the solution by pipetting up and down until complete solubilization. Protect from light.

**Fluorescent *in vitro* labeling with Poly(I:C) HMW Rhodamine**

The following protocol describes a method to detect Poly(I:C) HMW Rhodamine in murine macrophages RAW-Blue™ cells.

1. Prepare a Raw-Blue™ cell suspension (500,000 cells/ml) in DMEM with 10% (v/v) heat-inactivated fetal bovine serum .
2. In a 96-well plate, add 180 µl of the Raw-Blue™ cell suspension per well.
3. Stimulate cells with 10 ng-10 µg/ml Poly(I:C) HMW Rhodamine for 16 h.
4. Rinse cells with 1X PBS in order to remove free Poly(I:C) Rhodamine.
5. Analyze fluorescent labeling using one of the applications listed. Typical results are shown in figure 1.

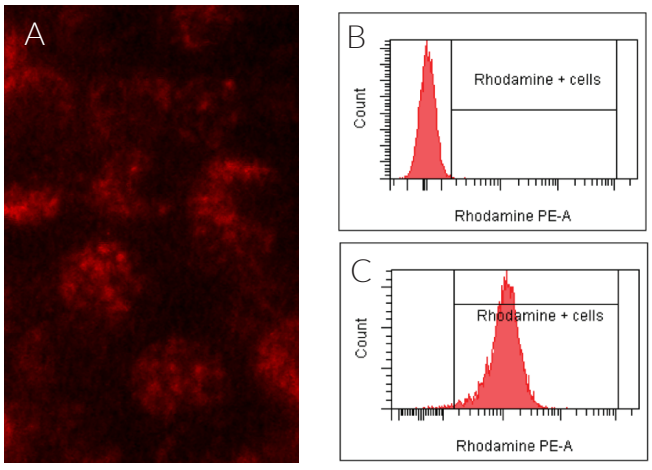


**Figure 1.** Intracellular fluorescent labeling of RAW-Blue™ (**A**) cells following a 16 h incubation with 10 µg/ml Poly(I:C) HMW Rhodamine (100,000 cells/well of a 96-well plate). Facs analysis of RAW-Blue™ cells following a 16 h incubation with (**B**) 10 µg/ml Poly(I:C) HMW and (**C**) 10 µg/ml Poly(I:C) HMW Rhodamine (100,000 cells/well of a 96-well plate).

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### TLR3 activation with Poly(I:C) HMW Rhodamine

Poly(I:C) HMW Rhodamine-induced TLR3 activation can be monitored using TLR3 reporter cell lines, such as HEK-Blue™ hTLR3 cells. These cells are designed for studying the stimulation of hTLR3 by monitoring the activation of NF-κB. Stimulation with a TLR3 ligand activates NF-κB and AP-1 which induces the production of SEAP. Levels of SEAP can be easily determined with QUANTI-Blue™ Solution (a detection medium that turns purple/blue in the presence of alkaline phosphatase).

1. Prepare a HEK-Blue™ hTLR3 cell suspension (250,000 cells/ml) in DMEM with 10% (v/v) heat-inactivated fetal bovine serum.
2. In a 96-well plate, add 180 µl of the HEK-Blue™ hTLR3 cell suspension per well.
3. Stimulate cells with 10 ng-10 µg/ml Poly(I:C) HMW Rhodamine for 6 to 24 h.
4. Determine Poly(I:C) HMW Rhodamine stimulation on TLR3 by assessing SEAP reporter expression using a SEAP detection medium, such as QUANTI-Blue™ Solution or HEK-Blue™ Detection.

A typical stimulation curve is given in figure 2.

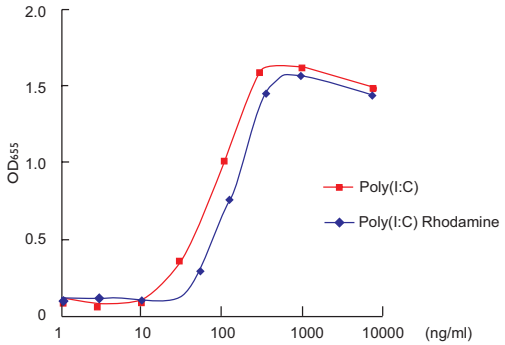


**Figure 2.** HEK-Blue™ hTLR3 cells were stimulated with increasing concentrations of Poly(I:C) HMW and Poly(I:C) HMW Rhodamine (50,000 cells/well of a 96-well plate). After 18h incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™ Solution.

## RELATED PRODUCTS

| Product | Description | Cat. Code |
|---|---|---|
| RAW-Blue™ cells | Macrophage reporter cells | raw-sp |
| HEK-Blue™ hTLR3 cells | Human TLR3 reporter cells | hkb-htlr3 |
| HEK-Blue™ Detection | SEAP detection medium | hb-det2 |
| QUANTI-Blue™ Solution | SEAP detection reagent | rep-qbs |
| Poly(I:C) HMW | TLR3 ligand | tlrl-pic |
| Poly(I:C) HMW Fluorescein | Fluorescein labeled Poly(I:C) | tlrl-picf |
| Poly(I:C) HMW Biotin | Biotin labeled Poly(I:C) | tlrl-picb |
| Poly(A:U) | TLR3 ligand | tlrl-pau |