

# Poly(I:C) LMW Rhodamine

## Low Molecular Weight

Rhodamine labeled synthetic analog of dsRNA; TLR3 ligand

Catalog code: tlr1-piwr

<https://www.invivogen.com/polyic-lmw-rhodamine>

For research use only

Version 21A27-MM

## PRODUCT INFORMATION

### Contents

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

### Storage and stability

- Poly(I:C) LMW Rhodamine is shipped at room temperature. Lyophilized product can be stored at -20°C for up to 6 months. Protect from light.
- Upon resuspension, prepare aliquots of Poly(I:C) LMW Rhodamine and store 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) LMW Rhodamine was chemically labeled by covalent coupling of a rhodamine probe containing a reactive alkylating group. This confers fluorescent properties to poly(I:C) LMW with a slight reduction of TLR3 recognition.

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)<sup>1,2</sup>. 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)<sup>3</sup>. 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<sup>4</sup>. 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)<sup>5</sup>.

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) LMW 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 100 ng-10 µg/ml Poly(I:C) LMW Rhodamine.

1. Add 100 µl of the endotoxin-free water (provided) to 10 µg Poly(I:C) LMW Rhodamine 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) LMW Rhodamine

The following protocol describes a method to detect Poly(I:C) LMW 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 100 ng-10 µg/ml Poly(I:C) LMW Rhodamine for 16 h.
4. Rinse cells with phosphate-buffered saline (PBS) in order to remove free Poly(I:C) LMW Rhodamine.
5. Analyze fluorescent labeling using one of the applications listed.

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

Poly(I:C) LMW Rhodamine-induced can be used to activate TLR3 in cells such as HEK-Blue™ TLR3 cells. These cells were transfected with the human TLR3 gene and an NF-κB-inducible SEAP (secreted alkaline phosphatase) reporter gene.

1. Prepare a HEK-Blue™ hTLR3 cell suspension (250,000 cells/ml).
2. Add 180 µl of the cell suspension per well of a 96-well plate.
3. Stimulate cells with 30 ng-10 µg/ml Poly(I:C) LMW Rhodamine for 6 to 24 hours.
4. Determine TLR3 activation by assessing reporter gene expression using QUANTI-Blue™ Solution or HEK-Blue™ Detection.

## 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) LMW	TLR3 ligand	tlr1-picw
Poly(A:U)	TLR3 ligand	tlr1-pau

## 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

E-mail: [info@invivogen.com](mailto:info@invivogen.com)

 **InvivoGen**  
www.invivogen.com