Poly(I:C) HMW Fluorescein

High Molecular Weight (HMW)

Fluorescein-labeled synthetic analog of dsRNA - TLR3 ligand

Catalog # tlrl-picf

For research use only

Version # 12F07-MM

PRODUCT INFORMATION

Content:

- 10 µg Poly(I:C) HMW Fluorescein
- 2 ml sterile endotoxin-free water

Storage:

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

Quality control:

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

Spectral Properties of Fluorescein

Excitation λ max: 492 nm
Emission λ max: 518 nm

DESCRIPTION

Poly(I:C) HMW Fluorescein was chemically labeled by covalent coupling of a fluorescein probe containing a reactive alkylating group. This confers fluorescent properties to poly(I:C) HMW (High molecular weight) whilst retaining TLR3 recognition properties (see figure 2 overleaf).

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) HMW Fluorescein 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) HMW Fluorescein.

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

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

The following protocol describes a method to detect Poly(I:C) HMW Flurescein 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.
- In a 96-well plate, add 180 µl of the Raw-Blue™ cell suspension per well.
- Stimulate cells with 10 ng -10 µg/ml Poly(I:C) HMW Fluorescein for 16 h.
- Rinse cells with 1X PBS to remove free Poly(I:C) HMW Fluorescein.
- Analyze fluorescent labeling using one of the applications listed. Typical results are shown in figure 1.

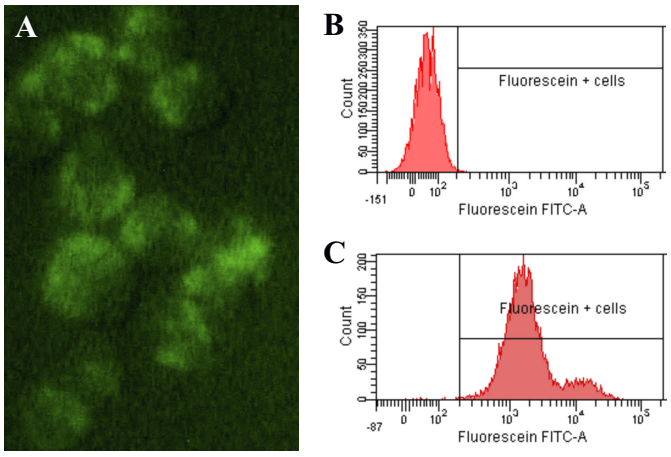

Figure 1. Intracellular fluorescent labeling of RAW-Blue™ (**A**) cells following a 16h incubation with 10 µg/ml Poly(I:C) HMW Fluorescein (100,000 cells/well of a 96-well plate). Facs analysis of RAW-Blue™ cells following a 16h incubation with (**B**) 10 µg/ml Poly(I:C) HMW and (**C**) 10 µg/ml Poly(I:C)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

TECHNICAL SUPPORT
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TLR3 activation with Poly(I:C) HMW Fluorescein

Poly(I:C) HMW Fluorescein-induced TLR3 activation can be monitored using TLR3 reporter cell lines, such as HEK-Blue™ hTLR3 cells. HEK-Blue™-hTLR3 cells were transfected with the human TLR3 gene and an NF-κB/AP-1-inducible SEAP (secreted alkaline phosphatase) reporter gene. Levels of SEAP can be easily determined with QUANTI-Blue™ (a detection medium that turns purple/blue in the presence of alkaline phosphatase). A typical stimulation curve obtained with QUANTI-Blue™ is given in figure 2.

- Prepare a HEK-Blue™ hTLR3 cell suspension (250,000 cells/ml) in DMEM, 4.5 g/l glucose, and 10% (v/v) heat-inactivated fetal bovine serum.
- In a 96-well plate, add 180 µl of the HEK-Blue™ hTLR3 cell suspension per well.
- Stimulate cells with 10 ng -10 µg/ml Poly(I:C) HMW Fluorescein for 6 to 24 h.
- Determine Poly(I:C) HMW Fluorescein stimulation on TLR3 by assessing reporter gene expression using QUANTI-Blue™ or HEK-Blue™ detection (see related products).

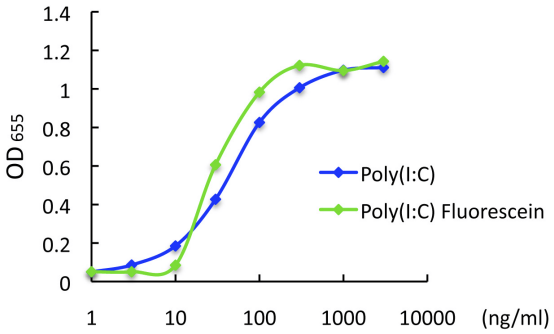

Figure 2. HEK-Blue™ hTLR3 cells were stimulated with increasing concentrations of Poly(I:C) HMW and Poly(I:C) HMW Fluorescein (50,000 cells/well of a 96-well plate). After an 18h incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™.

RELATED PRODUCTS

Product	Catalog Code
RAW-Blue™ cells	raw-sp
HEK-Blue™ hTLR3 cells	hkb-htlr3
QUANTI-Blue™	rep-qb1
Poly(I:C) HMW Rhodamine	tlrl-picr
Poly(I:C) HMW	tlrl-pic
Poly(A:U) (TLR3 ligand)	tlrl-pau