Poly(I:C) HMW

High Molecular Weight

Synthetic analog of dsRNA; TLR3 ligand

Catalog code: tlrl-pic, tlrl-pic-5

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

For research use only

Version 19D27-MM

PRODUCT INFORMATION

Contents

- Poly(I:C) HMW is provided lyophilized and is available in two sizes:
  - tlrl-pic: 10 mg
  - tlrl-pic-5: 50 mg
- Sterile endotoxin-free physiological water (NaCl 0.9%)
  - 10 ml with catalog code tlrl-pic
  - 2 x 25 ml with catalog code tlrl-pic-5

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at 4°C.
- Lyophilized product is stable for 1 year at 4°C when properly stored.
- Upon resuspension, prepare aliquots of Poly(I:C) HMW and store at 4°C or at -20°C. Resuspended product is stable for 1 month at 4°C and 1 year at -20°C. Avoid repeated freeze-thaw cycles.

Quality control:

- Absorbance spectrum
- TLR3 activity has been verified using HEK-Blue™ TLR3 cells
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using cellular assays.

BACKGROUND

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 NFkappaB by Toll-like receptor 3. Nature, 413(6857):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(5):1364-9. **3. Yamamoto M. *et al.*, 2003.** Role of Adaptor TRIF in the MyD88-Independent Toll-Like Receptor Signaling Pathway. 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. Review. **5. Kato H. et al., 2006.** Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. Nature. 441(7089):101-5.

PRODUCT DESCRIPTION

Polyinosinic-polycytidylic acid (usually abbreviated as poly(I:C) or poly(rI):poly(rC)) is a synthetic analog of double-stranded RNA (dsRNA), a molecular pattern associated with viral infection. Poly(I:C) activates the antiviral pattern recognition receptors TLR3, RIG-I/MDA5 and PKR, thereby inducing signaling via multiple inflammatory pathways, including NF-κB and IRF. High Molecular Weight Poly(I:C) comprises long strands of inosine poly(I) homopolymer annealed to strands of cytidine poly(C) homopolymer.

The average size of Poly(I:C) HMW is 1.5 to 8 kb.

METHODS

Preparation of stock solution (1 mg/ml)

Stimulation of TLR3 can be achieved with 30 ng - 10 µg/ml Poly(I:C).

- Add 10 ml of the endotoxin-free physiological water provided to the 10 mg Poly(I:C) vial or 50 ml to the 50 mg Poly(I:C) vial to obtain a solution at 1 mg/ml.
- Mix the solution by pipetting up and down.
- Heat the mixture for 10 minutes at 65-70°C. Allow the solution to cool for 1 hour at room temperature to ensure proper annealing.

TLR3 activation of TLR3 with Poly(I:C)HMW

Poly(I:C) HMW can be used to stimulate hTLR3 in 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™ (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).
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) HMW for 6 to 24 hours.
4. Determine poly(I:C) stimulation on TLR3 by assessing reporter gene expression using QUANTI-Blue™ Solution or HEK-Blue™ detection.

Note: InvivoGen provides also a low molecular weight poly(I:C), named poly(I:C)-LMW (see "Related Products"), with an average size of 0.2-1 kb that may activate the immune system differently.

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR3	hkb-htlr3
QUANTI-Blue™	rep-qb1
Poly(I:C)-LMW	tlrl-picw

TECHNICAL SUPPORT
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