Poly(I:C)-LMW

Low Molecular Weight

Synthetic analog of dsRNA - TLR3 ligand

Catalog # tlrl-picw, tlrl-picw-250

For research use only

Version # 11C21-MM

PRODUCT INFORMATION

Content:

Poly(I:C)-LMW is provided lyophilized and is available in two sizes:
- 25 mg: (catalog # tlrl-picw)
- 250 mg: (catalog # tlrl-picw-250)
- 10 ml or 2 x 25 ml sterile endotoxin-free physiological water (NaCl 0.9%)

Storage:

- Poly(I:C)-LMW is shipped at room temperature and should be stored at 4°C.
- Upon resuspension, prepare aliquots of Poly(I:C)-LMW and store at -20°C for long term storage. Store at 4°C for short term storage.
- Lyophilized product is stable 1 year at 4°C when properly stored. Resuspended product is stable 1 month at 4°C and 1 year at -20°C. Avoid repeated freeze-thaw cycles.

Quality control:

- Absorbance spectrum
- Gel retardation (Size: 0.2-1 kb)
- Human TLR3 (hTLR3) activity tested using HEK-Blue™ hTLR3 cells
- Endotoxin level: <0.05 EU/µg

DESCRIPTION

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. Biochem Biophys Res Commun, 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.** Tolllikereceptor 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. **6. Schindler U. & Baichwal VR., 1994**. Three NF-κB binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol, 14(9):5820-5831.

METHODS

Preparation of sterile stock solution (20 mg/ml)

Stimulation of TLR3 can be achieved with 30 ng - 10 µg/ml Poly(I:C)-LMW.
- Add 1.25 ml of the endotoxin-free physiological water provided to the 25 mg Poly(I:C)-LMW vial or 12.5 ml to the 250 mg Poly(I:C)-LMW vial to obtain a solution at 20 mg/ml.
- Mix the solution by pipetting up and down until complete solubilization.

Example of *in vitro* activation of TLR3 with Poly(I:C)-LMW using HEK-Blue hTLR3 cells

Poly(I:C)-LMW can be used to stimulate hTLR3 in HEK-Blue™ hTLR3 cells. HEK-Blue™-hTLR3 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). A typical stimulation curve is given in figure 1 overleaf.

- Prepare a HEK-Blue™ hTLR3 cell suspension (250,000 cells/ml) in DMEM, 4.5 g/l glucose, 10% (v/v) heat-inactivated fetal bovine serum (30 min at 56ºC), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine .
- In a 96-well plate, add 180 µl of the HEK-Blue™ hTLR3 cell suspension per well.
- Stimulate cells with 30 ng -10 µg/ml Poly(I:C)-LMW for 6 to 24 hours.
- Determine poly(I:C) stimulation on TLR3 by assessing reporter gene expression using QUANTI-Blue™ or HEK-Blue™ detection.

***Note:* InvivoGen provides also a high molecular weight poly(I:C) (see "Related Products"), with an average size of 1.5-8 kb that may activate the immune system differently.**

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue hTLR3	hkb-htlr3
QUANTI-Blue™	rep-qb1
HEK-Blue™ Detection	hb-det1
Poly(I:C)	tlrl-pic
Poly(A:U)	tlrl-pau
Poly(I:C) Rhodamine	tlrl-picr

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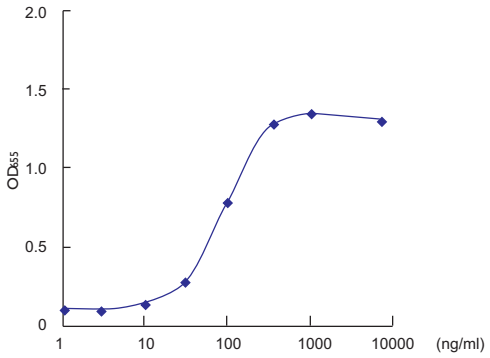

Figure 1. HEK-Blue™ hTLR3 cells were stimulated with increasing concentrations of Poly(I:C)-LMW. After 18h incubation, NF-κB-induced SEAP activity was assessed using QUANTI-Blue™.

Performance of this assay was validated under optimized conditions in a 96-well plate using QUANTI-Blue™.

Poly(I:C)-LMW EC50 = 82 +/- 8 ng/ml
Response Ratio = 10
Optimum cell number = 50,000 cells/well

TECHNICAL SUPPORT
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