Poly(I:C) HMW
High Molecular Weight
Synthetic analog of dsRNA - TLR3 ligand
Catalog # tlr1-pic, tlr1-pic-5
http://www.invivogen.com/polyic-hmw
For research use only
Version # 11C21-MM

PRODUCT INFORMATION

Content:
Poly(I:C) HMW is provided lyophilized and is available in two sizes:
- 10 mg: (catalog # tlr1-pic)
- 50 mg: (catalog # tlr1-pic-5)
- 10 ml or 2 x 25 ml sterile endotoxin-free physiological water (NaCl 0.9%)

Storage:
- Poly(I:C) HMW is shipped at room temperature and should be stored at 4°C.
- Upon resuspension, prepare aliquots of Poly(I:C) HMW 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: 1.5-8 kb)
- Human TLR3 (hTLR3) activity tested using HEK-Blue™ hTLR3 cells
- Endotoxin level: <0.001 EU/μg (measurement by kinetic chromogenic LAL assay)

DESCRIPTION
Polynosinic-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-xB and AP-14. 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 1 (RIG-1)5 and melanoma differentiation-associate gene 5 (MDA-5)6.

METHODS
Preparation of sterile 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.

Example of in vitro activation of TLR3 with Poly(I:C) using HEK-Blue hTLR3 cells
Poly(I:C) 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-kB. Stimulation with a TLR3 ligand activates NF-xB 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 figure 1 on the next page.

- 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 56°C), 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) 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 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

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog Code</th>
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</thead>
<tbody>
<tr>
<td>HEK-Blue™ hTLR3</td>
<td>hkb-hlr3</td>
</tr>
<tr>
<td>QUANTI-Blue™</td>
<td>rep-qb1</td>
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<tr>
<td>HEK-Blue™ Detection</td>
<td>hbp-det1</td>
</tr>
<tr>
<td>Poly(I:C)-LMW</td>
<td>tlr1-picw</td>
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<tr>
<td>Poly(A-U)</td>
<td>tlr1-pau</td>
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<tr>
<td>Poly(I:C) Rhodamine</td>
<td>tlr1-pier</td>
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</tbody>
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Figure 1. HEK-Blue™ hTLR3 cells were stimulated with increasing concentrations of Poly(I:C). 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) EC50 = 70 +/- 10 ng/ml
Response Ratio = 15
Optimum cell number = 50,000 cells/well