Poly(I:C) LMW
Low Molecular Weight
Synthetic analog of dsRNA; TLR3 ligand
Catalog code: tlrl-picw, tlrl-picw-250
https://www.invivogen.com/polyic-lmw

For research use only
Version 19D27-MM

PRODUCT INFORMATION

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

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) LMW 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.

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)\(^1\). 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)\(^5\).


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.

TLR3 activation of TLR3 with Poly(I:C) LMW
Poly(I:C)LMW 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) LMW for 6 to 24 hours.
4. 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) HMW (see
"Related Products"), with an average size of 1.5-8 kb that may activate the
immune system differently.

EC\(_{50}\) = 82 +/- 8 ng/ml

Figure 1. A typical stimulation curve. 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™.

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-htlr3</td>
</tr>
<tr>
<td>QUANTI-Blue™</td>
<td>rep-qb1</td>
</tr>
<tr>
<td>Poly(I:C) HMW</td>
<td>tlrl-picw</td>
</tr>
<tr>
<td>Poly(A:U)</td>
<td>tlrl-pau</td>
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</tbody>
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