Poly(I:C) HMW Biotin

High Molecular Weight (HMW)

Biotin-labeled synthetic analog of dsRNA; TLR3 ligand

Catalog code: tlrl-picb

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

For research use only

Version 20I14-MM

PRODUCT INFORMATION

Contents

- 10 µg Poly(I:C) HMW Biotin
- 1.5 ml endotoxin-free water

Storage and storage

- Poly(I:C) HMW Biotin is shipped at room temperature and can be stored at -20°C for up to 6 months.
- Upon resuspension, prepare aliquots of Poly(I:C) HMW Biotin and store at -20°C. Resuspended product is stable for 3 months at -20°C. Avoid repeated freeze-thaw cycles.

Quality control:

- TLR3 activity has been verified using HEK-Blue™ hTLR3 cells.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using cellular assays.
- Biotin coupling has been validated by flow cytometry

DESCRIPTION

Poly(I:C) HMW Biotin is a biotin-labeled Toll-like receptor 3 (TLR3) agonist. It was chemically produced by covalent coupling of biotin with high molecular weight polyinosinic-polycytidylic acid (poly(I:C) HMW). Poly(I:C) HMW, with an average size of 1.5-8 kb, comprises long strands of inosine poly(I) homopolymer annealed to strands of cytidine poly(C) homopolymer.

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 interferon (IFN) production[1]. Depending on its location in the cell, poly(I:C) can activate distinct pattern recognition receptor (PRR) pathways: endosomal poly(I:C) activates TLR3[2], whereas cytosolic poly(I:C) activates retinoic acid-inducible protein I (RIG-I)[3], melanoma differentiation-associate gene 5 (MDA-5)[4], and protein kinase RNA-activated (PKR)[5]. Activation of these PRRs results in the induction of multiple signaling pathways, including NF-κB and IFN regulatory factors (IRFs). Importantly, the labeled ligand retains the biological activity of poly(I:C). Each lot is thoroughly tested to ensure the absence of lot-to-lot variation.

1. Kawasaki T. & Kawai T., 2014. Toll-like receptor signaling pathways. Front Immunol. 5:461. **2. Alexopoulou L. *et al.*, 2001.** Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature, 413:732-8. **3. Kawai T. & Akira S., 2008.** Toll-like receptor and RIG-I-like receptor signaling. Ann N Y Acad Sci. 1143:1-20. **4. McCartney S. *et al.*, 2009.** Distinct and complementary functions of MDA5 and TLR3 in poly(I:C)-mediated activation of mouse NK cells. J Exp Med. 206(13):2967-76. **5. Lemaire P.A. *et al.*, 2008.** Mechanism of PKR Activation by dsRNA. J Mol Biol. 381(2):351-60. **6. Hasan M. *et al*, 2011.** Antimicrobial peptides inhibit polyinosinic-polycytidylic acid-induced immune responses. J Immunol. 187(11):5653-9. **7. Sugimoto N. *et al.*, 2014.** Helicase proteins DHX29 and RIG-I cosense cytosolic nucleic acids in the human airway system. PNAS. 111(21):7747-52.

APPLICATIONS

Poly(I:C) HMW Biotin can be used for various applications; including histochemical staining, cytometry, fluorescence microscopy, and ligand binding assays[6,7].

METHODS

Preparation of sterile stock solution (100 µg/ml)

1. Add 100 µl of the endotoxin-free water provided to the 10 µg Poly(I:C) HMW Biotin vial to obtain a solution at 100 µg/ml.
2. Homogenize the solution by pipetting up and down until the product is completely dissolved.

Working concentration: 30 ng -1 µg/ml

TLR3 activation with Poly(I:C) HMW Biotin

Poly(I:C) HMW Biotin-induced TLR3 activation can be monitored using TLR3 reporter cell lines, such as HEK-Blue™ hTLR3 cells. These cells were transfected with the human TLR3 gene and an NF-κB-inducible SEAP (secreted alkaline phosphatase) reporter gene. Levels of SEAP can be easily determined with QUANTI-Blue™ Soultion, a SEAP detection reagent.

For more information regarding the HEK-Blue hTLR3 cells please visit: https://www.invivogen.com/hek-blue-htlr3.

1. Prepare a HEK-Blue™ TLR3 cell suspension (280,000 cells/ml) in growth medium without Normocin™.
2. In a 96-well plate, add 180 µl of the HEK-Blue™ TLR3 cell suspension per well.
3. Stimulate cells with 30 ng -1 µg/ml Poly(I:C) HMW Biotin for 6 to 24 h.
4. Determine Poly(I:C) HMW Biotin stimulation of TLR3 by assessing SEAP expression using QUANTI-Blue™ Solution.

Note: Alternatively, HEK-Blue™ Detection can be used to assess SEAP expression.

RELATED PRODUCTS

Product	Description	Cat. Code
HEK-Blue™ hTLR3 cells	Human TLR3 reporter cells	hkb-htlr3
HEK-Blue™ mTLR3 cells	Murine TLR3 reporter cells	hkb-mtlr3
HEK-Blue™ Detection	SEAP detection reagent	hb-det2
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs
Poly(A:U)	TLR3 ligand	tlrl-pau
Poly(I:C) HMW	TLR3 ligand	tlrl-pic
Poly(I:C) HMW Fluorescein	Fluorescein-labeled poly(I:C)	tlrl-picf
Poly(I:C) HMW Rhodamine	Rhodamine-labeled poly(I:C)	tlrl-picr

TECHNICAL SUPPORT
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