Poly(I:C)(HMW)/LyoVec™

RIG-I/MDA-5 Ligand

Catalog code: tlrl-piclv

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

For research use only

Version 25C25-MM

PRODUCT INFORMATION

Contents

• 4 x 25 µg lyophilized poly(I:C)(HMW)/LyoVec™ 1:6 ratio (w/w)

Note: Each vial contains 25 µg of poly(I:C)-HMW complexed with 150 µg LyoVec™. Poly(I:C) HMW (high molecular weight) has an average size of 1.5-8 kb.

• 10 ml endotoxin-free water

Storage and stability

- Poly(I:C) (HMW)/LyoVec™ is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, store product at 4 °C. Resuspended product is stable for 1 week at 4 °C.

Quality control

• The biological activity has been tested using cellular assays.

• 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. Poly(I:C) induces a strong innate immune response initiated by two types of pattern recognition receptors (PRRs): the Toll-like receptors (TLRs) and the RIG-I-like receptors (RLRs)[1]. The TLR family consists of more than 10 members expressed on the cell surface membrane or endosomes. The RLRs form a family of cytoplasmic RNA helicases that includes RIG-I and MDA-5. Naked poly(I:C) is recognized by TLR3 whereas transfected poly(I:C) is sensed by RIG-I/MDA-5 in a cell-type-specific manner[2,3].

Poly(I:C) (HMW)/LyoVec™ are preformed complexes between poly(I:C) and the transfection reagent LyoVec™. These complexes induce the activation of the RIG-I/MDA-5 signaling pathway at concentrations ranging from 100 ng to 1 µg/ml in InvivoGen's RLR reporter cells.

1. Kawai T. & Akira S., 2007. Antiviral signaling through pattern recognition receptors. J Biochem. 141(2):137-45. **2. Gitlin L. *et al.*, 2006.** Essential role of mda-5 in type I IFN responses to polyriboinosinic:polyribocytidylic acid and encephelamyocarditis picornavirus. PNAS 103(22):8459-8464. **3. Kato H. *et al.*, 2005.** Cell type-specific involvement of RIG-I in antiviral response. Immunity. 23(1):19-28.

METHODS

Preparation of stock solution (50 µg/ml)

• Add 500 µl endotoxin-free water (provided) and mix gently. Allow at least 15 minutes to resuspend the product.

Note: The suspension may contain floating fine particles.

RIG-I/MDA-5 stimulation

A549-Dual™ cells express RIG-I, MDA-5, and an IFN regulatory factor-inducible Lucia luciferase reporter gene which provides a simple method to monitor activation of IFN signaling.

1. Resuspend poly(I:C) (HMW)/LyoVec™ as described above.
2. Add 20 µl of poly(I:C) (HMW)/LyoVec™ at different concentrations (100 ng to 1 µg/ml) per well of a flat-bottom 96-well plate.

Notes:

• *At final concentrations higher than 1 µg/ml, some cytoxicity may be observed.*

• *Naked poly(I:C) HMW may be used as negative control.*

3. To each well containing poly(I:C) (HMW)/LyoVec™, add 180 µl of an A549-Dual™ cell suspension (50,000 cells per well).
4. Incubate for 18-24 hours at 37 °C.
5. Determine poly(I:C) (HMW)/LyoVec™ stimulation of RIG-I/MDA-5 by assessing Lucia luciferase reporter gene expression using QUANTI-Luc™ 4 Lucia/Gaussia.

RELATED PRODUCTS

Products	Catalog Code
3p-hpRNA	tlrl-hprna
5'ppp-dsRNA	tlrl-3prna
A549-Dual™ Cells	a549d-nfis
LyoVec™	lyec-1
Poly(I:C) HMW	tlrl-pic
QUANTI-Luc™ 4 Lucia/Gaussia	rep-qlc4lg1

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