Poly(I:C)-HMW/LyoVec™

RIG-I/MDA-5 Ligand

Catalog code: tlrl-piclv

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

For research use only

Version # 17L04-MM

PRODUCT INFORMATION

Content:

• 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)/LyoVec™ complexes are provided lyophilized and shipped at room temperature. Store at -20 °C. Lyophilized product is stable for 1 year 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 HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

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)/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 in C57/WT MEFs

C57/WT murine embryonic fibroblasts (MEFs) were isolated from embryos under C57BL/6 background and immortalized with the SV40 large antigen. They stably express a SEAP reporter gene inducible by NF-κB and IRF3/7 providing a convenient method to monitor the activation of these transcription factors upon stimulation with poly(I:C)-HMW/LyoVec™ complexes.

1. Prepare a C57/WT cell suspension at ~415,000 cells/ml.
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. Add 180 µl of cell suspension (~75,000 cells) per well.
4. Incubate the plate at 37°C in a 5% CO_2 incubator for 18-24 h.
5. Monitor SEAP production using a SEAP detection assay such as QUANTI-Blue™.

RELATED PRODUCTS

Products	Catalog Code
3p-hpRNA	tlrl-hprna
5'ppp-dsRNA	tlrl-3prna
LyoVec™	lyec-1
Poly(I:C) HMW	tlrl-pic
QUANTI-Blue™	rep-qb1
RLR Reporter Cells	
A549-Dual™ Cells	a549d-nfis
A549-Dual™ KO-RIG-I Cells	a549d-korigi
B16-Blue™ IFN-α/β Cells	bb-ifnt1
HEK-Lucia™ RIG-I Cells	hkl-hrigi
C57/WT MEFs	mef-c57wt
RAW-Lucia™ ISG Cells	rawl-isg
THP1-Blue™ ISG Cells	thp-isg

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