5'ppp-dsRNA/LyoVec™

5' triphosphate double-stranded RNA complexed with LyoVec™; RIG-I Ligand

Catalog code: tlrl-3prnalv, tlrl-3prnalv-100

https://www.invivogen.com/5-ppp-dsrna-lyovec

For research use only

Version 18J26-MM

PRODUCT INFORMATION

Contents

• 5'ppp-dsRNA/LyoVec™ is available in two quantities:
- 25 µg 5'ppp-dsRNA/LyoVec™ cat code: tlrl-3prnalv
- 100 µg (4 x 25µg) 5'ppp-dsRNA/LyoVec™ cat code: tlrl-3prnalv-100

***Note:* Each vial contains 25 µg of 5'ppp-dsRNA complexed with 50 µg of LyoVec™.**

• Sterile endotoxin-free water, 1.5 ml with #tlrl-3prnalv and 10 ml with #tlrl-3prnalv-100

Sequence

5'- pppGCAUGCGACCUCUGUUUGA -3' (19 mer)
3'- CGUACGCUGGAGACAAACU -5' (19 mer)

5' triphosphate double stranded RNA is obtained by hybridization of one 5' triphosphate single-stranded 19-mer phosphodiester RNA with its complementary strand (non-triphosphatase). Each strand is chemically synthesized by solid-phase synthesis and purified by reverse phase HPLC.

Storage and stability

• 5'ppp-dsRNA/LyoVec™ is provided lyophilized and shipped at room temperature. Store lyophilized product at -20°C. Lyophilized product is stable for 12 months when properly stored.

• Upon resuspension, store 5'ppp-dsRNA/LyoVec™ at 4°C. Resuspended product is stable for 1 week when properly stored.

Quality control

- The biological activity has been verified using cellular assays.
- The absence of bacterial contamination, lipoproteins and endotoxins, has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

5' triphosphate double stranded RNA (5' ppp-dsRNA) is a synthetic ligand for retinoic acid-inducible protein I (RIG-I). RIG-I is a cytosolic pattern recognition receptor that senses pathogen-associated molecular patterns (PAMPs) on viral RNA and triggers an antiviral immune response by the activation of type-I interferons (IFNs)[1]. RIG-I shares the identification of the dsRNA structure with another sensor MDA-5 (recognition of poly(I:C)/LyoVec™) and is specifically activated by the uncapped 5' triphosphate moiety on viral RNA[2]. This triphosphate occurs during viral replication and is absent from most cytosolic self-RNA. A synthetic approach to the exact structure requirement to RIG-I recognition demonstrated that a short blunt double-stranded conformation containing a triphosphate at the 5' end is required[3-5]. 5' ppp-dsRNA is complexed with the cationic lipid LyoVec™ to facilitate its uptake.

1. Yoneyama M. & Fujita T., 2007. Function of RIG-I-like Receptors in Antiviral Innate Immunity. J. Biol. Chem. 282: 15315-8. **2. Hornung V. *et al.*, 2006.** 5'-Triphosphate RNA is the ligand for RIG-I. Science. 314(5801):994-7. **3. Schlee M. *et al.*, 2009.** Recognition of 5' triphosphate by RIG-I helicase requires short blunt double-stranded RNA as contained in panhandle of negative-strand virus. Immunity. 17;31(1):25-34. **4. Schmidt A. *et al.*, 2009.** .5'-triphosphate RNA requires base-paired structures to activate antiviral signaling via RIG-I. PNAS 106(29):12067-72. **5. Schlee M. & Hartmann G., 2010.** The chase for the RIG-I ligand - recent advances. Mol Ther. 18(7):1254-62.

METHODS

Stimulation of RIG-I can be achieved with 1-10 µg/ml of 5'ppp-dsRNA/LyoVec™.

- Add 500 µl sterile endotoxin-free water (provided) per vial of 25 µg of 5'ppp-dsRNA/LyoVec™. Mix gently. Allow at least 15 minutes for complete solubilization.
- Store at 4°C. Do not store for more than 1 week.

Induction of RIG-I in B16-Blue™ ISG cells

Induction of RIG-I with 5'ppp-dsRNA/LyoVec™ can be studied in a variety of cells expressing RIG-I. If your cell line does not naturally express the RIG-I gene, transfect with a plasmid expressing a RIG-I gene, such as pUNO-hRIG-I or pUNO-mRIG-I.

RIG-I activation can be easily monitored using InvivoGen's B16-Blue™ ISG cells, an IFN regulatory factor (IRF)-inducible secreted embryonic alkaline phosphatase (SEAP) reporter cell line. In these cells, activation of RIG-I triggers the secretion of IFNs that results in the production of SEAP. A protocol for the induction of RIG-I using B16-Blue™ ISG cells is given below:

- Resuspend 5'ppp-dsRNA/LyoVec™, as described above.
- Stimulate cells with 1-10 µg/ml 5'ppp-dsRNA/LyoVec™ for 16-24 hours. ***Note:* The use of 5'ppp-dsRNA Control (as a negative control) and Poly(I:C)/LyoVec™ (as a positive control) is highly recommended.**
- Monitor induction of RIG-I by measuring the levels of SEAP in the cell culture supernatant using QUANTI-Blue™, a SEAP detection reagent.

RELATED PRODUCTS

Product	Catalog Code
5'ppp-dsRNA Control/LyoVec™	tlrl-3prnaclv
B16-Blue™ ISG cells	bb-ifnabg
Poly(I:C) HMW/LyoVec™	tlrl-piclv
Poly(I:C) LMW/LyoVec™	tlrl-picwlv
pUNO1-hRIG-I (human gene)	puno1-hrigi
pUNO-mRIG-I (mouse gene)	puno-mrigi
QUANTI-Blue™ Solution	rep-qbs

TECHNICAL SUPPORT
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