3p-hpRNA/LyoVec™

5' triphosphate hairpin RNA complexed with LyoVec™; RIG-I ligand

Catalog code: tlrl-hprnalv, tlrl-hprnalv-100

https://www.invivogen.com/3p-hprnalv

For research use only

Version 20A13-NJ

PRODUCT INFORMATION

Contents

3p-hpRNA/LyoVec™ is available in two quantities:

- 25 µg 3p-hpRNA/LyoVec™ (cat. code: tlrl-hprnalv)
- 100µg (4 x 25 µg) 3p-hpRNA/LyoVec™ (cat. code: tlrl-hprnalv-100)

Note: Each vial contains 25 µg of 3p-hpRNA complexed with 50 µg of LyoVec™.

- Sterile endotoxin-free water: 1.5 ml with #tlrl-hprnalv, and 10 ml with #tlrl-hprnalv-100

Sequence

5'-pppGGAGCAAAAGCAGGGUGACAAAGACAUAAUGGAUCCAA ACACUGUGUCAAGCUUUCAGGUAGAUUGCUUUCUUU GGCAUGUCCGCAAAC- 3' (89 mer)

Note: 3p-hpRNA was prepared by in vitro transcription with T7 RNA polymerase. This sequence self-anneals to form secondary structures such hairpin or panhandle conformations.

Storage and stability

- 3p-hpRNA/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, prepare aliquots and store at 4 °C. Resuspended product is stable for 1 week at 4 °C.

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

3p-hpRNA is a 5' triphosphate hairpin RNA that was generated by *in vitro* transcription of a sequence from influenza A (H1N1) virus, a single-stranded negative-sense RNA virus[1,2]. This 89 mer RNA oligonucleotide contains an uncapped 5'triphosphate extremity and a double strand fragment. These structural features, which distinguish viral RNA from mammalian RNA, are recognized by retinoic acid-inducible gene I (RIG-I), the founding member of the RIG-I like receptor (RLR) family[3,4]. RLRs are cytosolic sensors responsible for the detection of viral RNA. Upon activation, RIG-I recruits the adaptor mitochondrial antiviral signaling protein (MAVS, or IPS-1/VISA/Cardif) then triggers signaling cascades that lead to the production of type I interferons and pro-inflammatory cytokines[5]. Importantly, 3p-hpRNA is a specific agonist of RIG-I; it does not activate other dsRNA sensors such as TLR3 and MDA-5. Of note, this ligand more potently induces RIG-I than the fully synthetic RIG-I ligand 5'ppp-dsRNA.

3p-hpRNA is complexed with the cationic lipid LyoVec™ to facilitate its cellular uptake.

METHODS

Preparation of 3p-hpRNA/LyoVec™ stock solution (50 µg/ml)

Note: As 3p-hpRNA is sensitive to electrostatic charges, we recommend using nitrile gloves (which dissipate electrostatic charge better than latex).

- Briefly centrifuge the vial before opening to dislodge any lyophilized material that may be dispersed on the wall or cap of the vial. Carefully open the vial lid to avoid any loss of product.
- Add 500 µl of sterile RNAse-free endotoxin-free water to 25 µg of 3p-hpRNA/LyoVec™. Mix gently. Allow at least 15 minutes for complete solubilization.

Note: Use sterile RNAse-free tubes and water to avoid product degradation.

Working concentration: 10 ng - 3 µg/ml depending on cell lines

RIG-I stimulation using 3p-hpRNA/LyoVec™ in A549-Dual™ cells

A549-Dual™ cells express RIG-I and an IFN regulatory factor (ISG)-inducible Lucia luciferase reporter gene which provides a simple method to monitor activation of IFN signaling. upon RIG-I stimulation. 3p-hpRNA must be delivered to the cytoplasm, for example by using a transfection agent, such as LyoVec™.

1. Prepare 3p-hpRNA/LyoVec™ stock solution as described above or thaw aliquots and bring them to room temperature.
2. Prepare a 1:20 dilution of the 3p-hpRNA/LyoVec™ complex using sterile RNAse-free endotoxin-free water
3. Add 20 µl of the diluted 3p-hpRNA/LyoVec™ complex to each well of a 96-well plate.
4. To each well containing 3p-hpRNA/LyoVec™ complex, add 180 µl of an A549-Dual™ cell suspension (50,000 cells per well).
5. Incubate for 16-24 hours at 37 °C.
6. Determine 3p-hpRNA stimulation of RIG-I by assessing Lucia luciferase reporter gene expression using QUANTI-Luc™, a luciferase detection reagent.

RELATED PRODUCTS

Product	Catalog Code
5'ppp-dsRNA/LyoVec™	tlrl-3prnalv
A549-Dual™ Cells	a549d-nfis
A549-Dual™ KO-MAVS Cells	a549d-komavs
A549-Dual™ KO-RIG-I Cells	a549d-korigi
QUANTI-Luc™	rep-qlc1

1. Rehwinkel J. *et al.*, 2010. RIG-I detects viral genomic RNA during negative-strand RNA virus infection. Cell. 140:397-408. **2. Liu G. *et al.*, 2015.** Influenza A Virus Panhandle Structure Is Directly Involved in RIG-I Activation and Interferon Induction. J Virol. 89(11):6067-79. **3. Hornung V. *et al.*, 2006.** 5'-triphosphate RNA is the ligand for RIG-I. Science. 314:994-7. **4. Gebhardt A. *et al.*, 2017.** Discrimination of Self and Non-Self Ribonucleic Acids. Journal of Interferon & Cytokine Research 37: 184-97. **5. Yoneyama M. *et al.*, 2015.** Viral RNA detection by RIG-I-like receptors. Curr Opin Immunol. 32:48-53.

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