5'ppp-dsRNA Control

Negative control for 5'ppp-dsRNA

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

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

For research use only

Version 18J26-MM

PRODUCT INFORMATION

Contents:

• 5'ppp-dsRNA Control is available in two quantities:
  - 25 µg 5'ppp-dsRNA Control: cat. code tlrl-3prnac
  - 100 µg (4 x 25µg) 5'ppp-dsRNA Control: cat. code tlrl-3prnac-100
• sterile endotoxin-free water, 2 ml with #tlrl-3prnac and 10 ml with #tlrl-3prnac-100.

Sequence of 5' ppp-dsRNA Control:

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

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

Storage and stability:

- 5'ppp-dsRNA Control is provided lyophilized and shipped at room temperature. Store lyophilized 5'ppp-dsRNA Control at -20°C. Lyophilized product is stable for 1 year when stored -20°C.
- Upon resuspension, prepare aliquots of 5'ppp-dsRNA Control and store at -20°C. Resuspended product is stable for 1 month at -20°C. Avoid repeated freeze-thaw cycles.

Quality control

- 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]. 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, 4]. 5'ppp-dsRNA Control does not contain the 5' triphosphate moiety, however, caution should be taken as dsRNA without 5' ppp can induce an IFN response in certain cell lines via non-RIG-I pathways, such as TLR7 in plasmacytoid dendritic cells[5].

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. et al., 2010.** The chase for the RIG-I lignad - Recent advances. Mol Ther. 18(7):1254-62

METHODS

Preparation of 5'ppp-dsRNA Control stock solution (0.1 µg/µl)

Note: As this product is sensitive to electrostatic charges, we highly recommend the use of nitrile gloves (which can dissipate electrostatic charge much better than latex) and to spin down the vial before opening it. Be aware of the volatility of the product.

- Add 250 µl of sterile RNAse-free endotoxin-free water to the 25 µg of 5'ppp-dsRNA Control. Mix gently until complete solubilization.

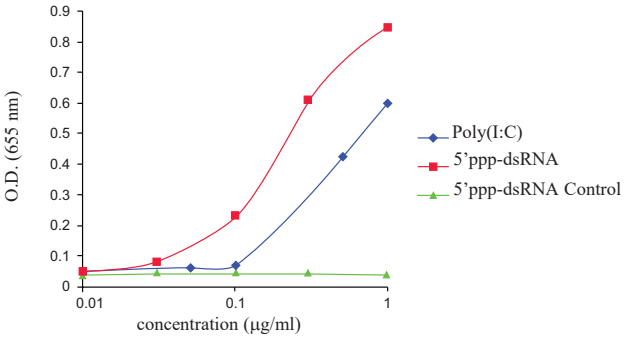
RIG-I Stimulation

Stimulation of RIG-I can be achieved with 5'ppp-dsRNA in cells expressing RIG-I. To achieve 5'ppp-dsRNA stimulation of RIG-I, the 5'ppp-dsRNA must be delivered intracellularly, for example by using a transfection agent, such as LyoVec™.

Note: 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.

Example of RIG-I stimulation using 5'ppp-dsRNA and LyoVec™ transfection reagent in B16-Blue™ ISG cells

RIG-I activation with 5'ppp-dsRNA can be easily monitored using B16 Blue™ ISG cells, an IFN regulatory factor (IRF)-inducible secreted embryonic alkaline phosphatase (SEAP) reporter cell line. Following transfection of the 5'ppp-dsRNA with LyoVec™ in B16-Blue™ ISG cells, recognition by murine RIG-I triggers the secretion of IFNs that results in the production of SEAP by activation of an IRF-inducible promoter. Levels of SEAP can be easily determined by colorimetric measurement using QUANTI-Blue™ (a detection medium that turns purple/blue in the presence of alkaline phosphatase).



RIG-I response to 5'ppp-dsRNA. B16-Blue™ ISG cells respond to stimulation with 5'ppp-dsRNA and poly(I:C). The 5'ppp-dsRNA control (non-phosphorylated) did not induce any response in B16-Blue™ ISG cells. Levels of SEAP were determined with QUANTI-Blue™ .

TECHNICAL SUPPORT
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Below is a protocol for determining RIG-I stimulation with 5'ppp-dsRNA. Please note that 5'ppp-dsRNA Control has no activity on RIG-I. Use 5'ppp-dsRNA control at the same concentration as 5'ppp-dsRNA.

Protocol

1. Rehydrate 5'ppp-dsRNA and LyoVec™ at the recommended concentrations. Bring LyoVec™ and 5' ppp-dsRNA to room temperature and gently vortex to homogenize before use.
Note: The use Poly(I:C) (as a positive control) is highly recommended.
2. In a sterile 1.5 ml microfuge tube at room temperature, mix 10 µl (1 µg) of 5'ppp-dsRNA stock solution (0.1 µg/µl) with 100 µl of LyoVec™. Mix gently.
3. Incubate at room temperature for 15 minutes to allow the formation of the complex.
4. Add 10 - 20 µl of 5'ppp-dsRNA & LyoVec™ complex to each well of a 96-well plate.
5. To each well containing 5'ppp-dsRNA & LyoVec™, add 180 µl of a B16-Blue™ ISG cell suspension (400,000 cells/ml) in RPMI.
6. Incubate for 24 hours at 37°C.
7. Determine 5'ppp-dsRNA stimulation of RIG-I by assessing SEAP reporter gene expression using QUANTI-Blue™.

RELATED PRODUCTS

Product	Catalog Code
5'ppp-dsRNA	tlrl-3prna
B16-Blue™ ISG cells	bb-ifnabg
LyoVec™	lyec-12
Poly(dA-dT)•Poly(dA-dT)	tlrl-pat
Poly(I:C)	tlrl-pic
Poly(I:C)/LyoVec™	tlrl-piclv
Poly(I:C) LMW	tlrl-picw
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

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