ORN 06/LyoVec™

GU-rich oligonucleotide complexed with LyoVec™

Catalog # tlrl-orn6

For research use only

Version # 10J26-MM

PRODUCT INFORMATION

Content

- 4 x 25 µg lyophilized ORN 06/LyoVec™
ORN 06 5'-UUGUUGUUGUUGUUGUUGUU-3' (20 mer)
- 10 ml endotoxin-free water

Storage and stability:

- ORN 06/LyoVec™ is provided lyophilized and shipped at room temperature. Store at -20°C. Lyophilized product is stable 1 year at -20˚C.
- Upon resuspension, store product at 4°C. Resuspended product is stable 1 week at 4°C.

BACKGROUND

TLR7 and TLR8 play an important role in the innate antiviral immunity by recognizing viral RNA in endocytic compartments. Single-stranded RNA (ssRNA) has been identified as the natural ligand of TLR7 and TLR8[1, 2]. ssRNA derived from HIV-1 or the influenza virus were shown to induce the production of proinflammatory cytokines in pDC. This induction was reproduced using U-rich single-stranded RNAs, such as polyU or GU-rich (ssRNA40) ODNs complexed with cationic lipids to protect them from degradation. TLR7/8 activation by viral RNA can be mimicked by short U-rich single-stranded RNAs (ssRNAs). According to Diebold *et al*., this activation is sequence-independent as long as the ssRNAs contain several uridines in close proximity[1]. However, recent studies suggest sequence-dependent recognition of U-rich ssRNAs by TLR7 and TLR8. They demonstrate that AU-rich and GU-rich oligoribonucleotides (ORNs) are capable of activating TLR8, whereas only GU-rich ORNs are able to stimulate TLR7[3]. InvivoGen provides a selection of ssRNAs/ORNs described in the literature. All ssRNAs/ORNs have been tested in 293 cells stably transfected with TLR7 or TLR8 to confirm their specificity.

ORN 06 contains 6 repeats of the UUGU sequence motif, identified as the minimal motif responsible for ssRNA40 immunoactivity[3].

REFERENCES

1. Diebold S. et al., 2006. Nucleic acid agonists for Toll-like receptor 7 are defined by the presence of uridine ribonucleotides. Eur. J. Immunol., 36:3256-67. **2. Heil F. *et al.*, 2004.** Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 5;303(5663):1526-9 **3. Forsbach A. et al., 2008.** Identification of RNA Sequence Motifs Stimulating Sequence-Specific TLR8-Dependent Immune Responses. J. Immunol., 180: 3729-38.

METHODS

Preparation of stock solution (50 µg/ml)

Stimulation of mouse TLR7 and human TLR8 can be achieved with 0.1-10 µg/ml ORN 06/LyoVec™ .

- Add 500 µl endotoxin-free water (provided) and mix gently. Allow at least 15 minutes for complete solubilization.

Human TLR8 / Mouse TLR7 stimulation

- Transfect your cell line with an NF-κB-inducible reporter plasmid, i.e. a plasmid carrying a reporter gene, such as SEAP or luciferase, under the control of an NF-κB-inducible ELAM-1 (E-selectin) promoter[3].

Note: InvivoGen provides pNiFty, a family of NF-κB-inducible reporter plasmids that can be transfected transiently (pNiFty) or stably (pNiFty2). pNiFty plasmids are available either with the SEAP or luciferase reporter genes (see Related Products).

If your cell line does not naturally express the mouse TLR7 or human TLR8 gene, cotransfect with a plasmid expressing either TLR gene, such as pUNO-mTLR7 or pUNO1-hTLR8b (see Related Products).

- Twenty-four to forty-eight hours after transfection, stimulate cells with 0.1-10 µg/ml ORN 06/LyoVec™ for 6 hours to 36 hours.
- Determine ORN 06/LyoVec™ stimulation on mouse TLR7 or human TLR8 by assessing reporter gene expression using the appropriate detection system.

RELATED PRODUCTS

Product	Catalog Code
pNiFty-Luc (Amp^R)	pnifty-luc
pNiFty-SEAP (Amp^R)	pnifty-seap
pNiFty2-Luc (Zeo^R)	pnifty2-luc
pNiFty2-SEAP (Zeo^R)	pnifty2-seap
pUNO-mTLR7 (mouse gene)	puno-mtlr7
pUNO1-hTLR8b (human gene)	puno1-htlr8b
293/hTLR8 (human gene)	293-htlr8
293/mTLR7 (mouse gene)	293-mtlr7
ORN 02/LyoVec™	tlrl-orn2
ssPolyU/LyoVec™	tlrl-lpu
ssRNA40/LyoVec™	tlrl-lrna40
ssRNA-DR/LyoVec™	tlrl-ssdr

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