ssRNA40/LyoVec™

Single-stranded GU-rich oligonucleotide complexed with LyoVec™

Catalog # tlrl-lrna40

For research use only

Version # 10J26-MM

PRODUCT INFORMATION

Content:

• 4x 25 µg lyophilized ssRNA40/LyoVec™ 1:2 ratio (w/w)

Note: Each vial contains 25 µg of ssRNA40 complexed with 50 µg LyoVec™.

ssRNA40 5'-GsCsCsCsGsUsCsUsGsUsUsGsUsGsUsGsAsCsUsC-3'
("s" depicts a phosphothioate linkage)

• 10 ml endotoxin-free water

Storage and stability:

- ssRNA40/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.

DESCRIPTION

ssRNA40 is a 20-mer phosphothioate protected single-stranded RNA oligonucleotide containing a GU-rich sequence[1]. ssRNA40 is complexed with the cationic lipid LyoVec™, to protect it from degradation and facilitate its uptake, and lyophilized to generate ssRNA40/LyoVec™. When complexed to cationic lipids, ssRNA can substitute for viral RNAs in inducing TNFα and IFNα production in peripheral blood mononuclear cells[1, 2]. Murine dendritic cells deficient for TLR7 failed to produce IFNα in response to ssRNA40, while the response to CpG-ODNs was unaffected, suggesting that TLR7 plays a critical role in viral ssRNA recognition[1]. In human cells, TLR8 was shown to be the key receptor for viral ssRNA, implying a species specificity difference in ssRNA recognition. During infection, some viral particles are degraded by the endosomal proteases, exposing the viral genome and allowing TLR7 and/or TLR8 signaling, which are known to occur in endosomes[3]. TLR7 and TLR8 can recognize both self and viral RNA but seem able to distinguish the presence of viral RNA by detecting their abnormal localization in the endosome rather than a particular RNA motif.

1. 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.
2. Diebold SS. *et al.*, 2004. Innate antiviral responses by means of TLR7-mediated recognition of single-stranded RNA. Science. 5;303(5663):1529-31
3. Heil F. *et al.*, 2003 The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncovers a strong relationship within the TLR7, 8 and 9 subfamily. Eur J Immunol. 33(11):2987-97.
4. Schindler U. & Baichwal VR., 1994. Three NF-κB binding sites in the human E-selectin gene required for maximal tumor necrosis factor alpha-induced expression. Mol Cell Biol, 14(9):5820-5831.

METHODS

Preparation of stock solution (50 µg/ml)

Stimulation of mouse TLR7 and human TLR8 can be achieved with 0.25-5 µg/ml ssRNA40/LyoVec™.

- Add 500 µl sterile 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[4].

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 0.25-5 µg/ml ssRNA40/LyoVec™ for 6 hours to 36 hours.

- Determine ssRNA40/LyoVec™ stimulation on mouse TLR7 or human TLR8 by assessing reporter gene expression using the appropriate detection system.

RELATED PRODUCTS

Products	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
293XL/hTLR8A (human gene)	293xl-htlr8
293XL/mTLR7 (mouse gene)	293xl-mtlr7

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