

***E. coli* RNA/LyoVec™**

Single stranded *E. coli* K12 RNA complexed with LyoVec™

Catalog # tlr1-ecrna

For research use only

Version # 10D01-MM

PRODUCT INFORMATION

Content:

- 4x 25 µg lyophilized *E. coli* RNA/LyoVec™ 1:2 ratio (w/w)

Note: Each vial contains 25 µg of *E. coli* K12 RNA complexed with 50 µg LyoVec™.

- 10 ml endotoxin-free water

Storage and stability:

- *E. coli* RNA/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

Total RNA derived from bacteria, but not from eukaryotic cells, induces high levels of IL-12 secretion in dendritic cells¹. This difference is explained by the fact that bacterial RNA contains significantly less nucleoside modifications than mammalian RNA². Examples of modified nucleosides found in mammalian RNAs are 5-methylcytidine, N6-methyladenosine, inosine and many 2'-O-methylated nucleosides. These nucleoside modifications have been reported to suppress the immunostimulatory effect of RNA³. Bacterial *E. coli* K12 RNA complexed with a cationic lipid (LyoVec™), to protect it from degradation and facilitate its uptake, induce the activation of NF-κB in HEK293 cells expressing TLR7 or TLR8.

1. Koski GK. *et al.*, 2004. Cutting edge: innate immune system discriminates between RNA containing bacterial versus eukaryotic structural features that prime for high-level IL-12 secretion by dendritic cells. *J Immunol.* 172(7):3989-93.

2. Kariko K. *et al.*, 2005. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity.* 23(2):165-75.

3. 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 TLR7 and TLR8 can be achieved with 1-10 µg/ml *E. coli* RNA/LyoVec™.

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

ssRNA-DR/LyoVec™ 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) promoter².

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 TLR7 or TLR8, cotransfect with a plasmid expressing either TLR gene, such as the pUNO plasmids (see Related Products).

- Twenty-four to forty-eight hours after transfection, stimulate cells with 1-10 µg/ml *E. coli* RNA/LyoVec™ for 6 hours to 36 hours.

- Determine *E. coli* RNA/LyoVec™ stimulation on TLR7 or 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-hTLR7 (human gene)	puno-htlr7
pUNO1-hTLR8b (human gene)	puno1-htlr8b
293/hTLR7 (human gene)	293-htlr7
293/hTLR8 (human gene)	293-htlr8

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