

***E. coli* ssDNA/LyoVec™**

Single-stranded sheared *E. coli* DNA complexed with LyoVec™; a TLR9 ligand

Catalog code: tlr1-ssec

<https://www.invivogen.com/ecoli-ssdna-lyovec>

For research use only

Version 21D16-MM

PRODUCT INFORMATION

Contents

- 4 x 50 µg lyophilized *E. coli* ssDNA/LyoVec™ 1:2 ratio (w/w)

Note: Each vial contains 50 µg of *E. coli* K12 ssDNA complexed with 100 µg LyoVec™.

- 10 ml endotoxin-free water

Storage and stability

- *E. coli* ssDNA/LyoVec™ is shipped at room temperature. Upon receipt, store at -20°C. Lyophilized product is stable for 1 year at -20°C.
- Upon resuspension, store product at 4°C. Resuspended product is stable for 1 week at 4°C.

Quality control

- The biological activity has been tested using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 cells and HEK-Blue™ TLR4 cells.

DESCRIPTION

E. coli ssDNA/LyoVec™ is a Toll-like receptor 9 (TLR9) ligand. It comprises *E. coli* ssDNA an ultrapure, endotoxin-free preparation of bacterial single-stranded DNA (ssDNA) which is complexed with the cationic lipid-based transfection reagent LyoVec™. This allows a better internalization of the immunostimulatory DNA to the acidic endosomal compartment where TLR9 is expressed.

TLR9 specifically recognizes CpG DNA that is unmethylated and single-stranded (ss). Upon recognition, it initiates a signaling cascade leading to the activation of the transcription factors NF-κB, AP-1, and interferon regulatory factor 7 (IRF7) with the subsequent production of pro-inflammatory cytokines. Methylation of the cytosine within the CpG motif strongly reduces the affinity of TLR9^{1,2}. In addition, double-stranded (ds) CpG DNA is a much weaker stimulator of TLR9 compared to its ss counterpart¹. Unlike mammalian DNA, bacterial DNA is rich in unmethylated CpG motifs and thus activates TLR9. InvivoGen provides bacterial ssDNA as sheared single-stranded DNA fragments produced by treating genomic *E. coli* DNA with ultrasound followed by heat denaturation. These fragments are complexed with LyoVec™ to allow penetration of the DNA in the cells. *E. coli* ssDNA/LyoVec™ activates TLR9 similarly to CpG-ODNs in a species-independent manner. Of note, the ability of *E. coli* ssDNA/LyoVec™ to induce an inflammatory response has been described in the literature³.

1. Rutz M. *et al.*, 2004. Toll-like receptor 9 binds single-stranded CpG-DNA in a sequence- and pH-dependent manner. *Eur J Immunol*. 16:17-22. 2. Cornелиe S. *et al.*, 2004. Direct evidence that toll-like receptor 9 (TLR9) functionally binds plasmid DNA by specific cytosine-phosphate-guanine motif recognition. *J Biol Chem*. 279:15124-9. 3. Yu S. *et al.*, 2014. TLR sorting by Rab11 endosomes maintains intestinal epithelial-microbial homeostasis. *EMBO J*. 33:1882-95.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-34-80

E-mail: info@invivogen.com

METHODS

Preparation of stock solution (50 µg/ml)

- Add 1 ml endotoxin-free water (provided) per vial and mix gently. Allow at least 15 minutes for complete solubilization.

Working concentrations: 500 ng/ml - 5 µg/ml for TLR9

TLR9 stimulation using *E. coli* ssDNA/LyoVec™

E. coli ssDNA/LyoVec™ can be used to stimulate in cells expressing the TLR9 gene, such as in HEK-Blue™ TLR9 cells. These cells were transfected with the TLR9 gene and an NF-κB-inducible secreted alkaline phosphatase (SEAP) reporter gene. Levels of SEAP can be easily determined with HEK-Blue™ Detection, a SEAP detection medium that turns purple/blue in the presence of alkaline phosphatase.

For more information, <https://www.invivogen.com/hek-blue-tlr9>

1. Dispense 20 µl of *E. coli* ssDNA/LyoVec™ (500 ng/ml - 5 µg/ml final concentration) per well of a 96-well plate.
2. Prepare a suspension of HEK-Blue™ TLR9 cells in HEK-Blue™ Detection medium.
3. Immediately add 180 µl of the cell suspension to each *E. coli* ssDNA/LyoVec™-containing well.
4. Incubate the plate at 37°C in a CO₂ incubator for 6-24 hours.
5. Determine SEAP levels using a spectrophotometer at 620-655 nm.

RELATED PRODUCTS

Product	Description	Cat.Code
dsDNA-EC	dsDNA from <i>E. coli</i> K12	tlrl-ecdna
HEK-Blue™ Detection	SEAP detection medium	hb-det2
HEK-Blue™ hTLR9 Cells	Human TLR9 reporter cells	hkb-htlr9
HEK-Blue™ mTLR9 Cells	Murine TLR9 reporter cells	hkb-mtlr9
ODN 1585	Murine TLR9 ligand	tlrl-1585
ODN 2006	Human TLR9 ligand	tlrl-2006