

dsDNA-EC

Double-stranded genomic DNA from *E. coli* K12 - CDS & TLR9 ligand

Catalog # tlrl-ecdna

For research use only

Version # 16J13-MT

PRODUCT INFORMATION

Content:

- 200 µg lyophilized dsDNA-EC
- 1.5 ml endotoxin-free water

Storage:

- dsDNA-EC is shipped at room temperature and should be stored at -20°C. Lyophilized product is stable for 1 year when properly stored.
- Upon resuspension, prepare aliquots of dsDNA-EC and store at -20°C. Resuspended product is stable for 1 year when properly stored. Avoid repeated freeze-thaw cycles.

Quality Control:

- Endotoxin levels: <0.01 EU/µg

Working concentrations:

- 30 ng - 1 µg/ml transfected dsDNA-EC to activate CDSs
- 1 µg/ml transfected dsDNA-EC to activate TLR9

DESCRIPTION

dsDNA-EC is an ultrapure preparation of double-stranded genomic DNA (dsDNA) from *Escherichia coli* K12. Transfected *E. coli* dsDNA is sensed by the cytosolic DNA sensor (CDS) cGMP-AMP synthase (cGAS) leading to the production of type I interferons (IFN) through the STING-TBK1-IRF3 pathway^{1,2}. It is also recognized by the endosomal receptor TLR9, due to the presence of unmethylated CpG motifs³, and triggers the NF-κB pathway⁴.

InvivoGen has developed several stable reporter cell lines to facilitate the study of the innate immune response to *E. coli* dsDNA. Such cell lines include THP1-Blue™ ISG cells and HEK-Blue™ TLR9 cells. THP1-Blue™ ISG cells, which are derived from the human monocytic THP-1 cell line and stably express an IRF-inducible Lucia luciferase reporter gene, can be used to study the activation of the cGAS-IRF3 pathway. The HEK-Blue™ TLR9 cells, which are HEK293-derived cells that stably overexpress the TLR9 gene and an NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene, are designed for the study of the TLR9-NF-κB pathway. Both cell lines respond to transfected dsDNA-EC (see “Methods”).

1. Li XD, *et al.*, 2013. Pivotal roles of cGAS-cGAMP signaling in antiviral defense and immune adjuvant effects. *Science*. 341(6152):1390-4. 2. Ma Z, *et al.*, 2015. Modulation of the cGAS-STING DNA sensing pathway by gammaherpesviruses. *PNAS* 112(31):E4306-15. 3. Hemmi H, *et al.*, 2000. A Toll-like receptor recognizes bacterial DNA. *Nature*. 408(6813):740-5. 4. Ramirez-Ortiz ZG, *et al.*, 2008. Toll-like receptor 9-dependent immune activation by unmethylated CpG motifs in *Aspergillus fumigatus* DNA. *Infect Immun*. 76(5):2123-9.

METHODS

Preparation of stock solution (1 mg/ml)

- Add 200 µl endotoxin-free water to 200 µg dsDNA-EC. Mix by pipetting up and down.
- Use immediately or prepare aliquots and store at -20°C.

Extemporaneous preparation of dsDNA-EC/cationic lipid complex

To facilitate the intracellular delivery, complex dsDNA-EC with a cationic lipid transfection agent, such as LyoVec™.

- Rehydrate dsDNA-EC as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring dsDNA-EC and LyoVec™ to room temperature before use.
- In a sterile 1.5 ml microfuge tube, mix 1 µg dsDNA-EC with 100 µl of LyoVec™. Homogenize gently.
- Incubate at room temperature for 15 minutes to allow the formation of the complex. Do not keep complex for more than 1 day.

Induction of IRF3 in THP1-Blue ISG cells

Activation of the cGAS-IRF3 pathway by transfected dsDNA-EC can be monitored in THP1-Blue™ ISG cells.

- Prepare dsDNA-EC/LyoVec™ complex, as described above.
- Stimulate cells with 30 ng/ml to 1 µg/ml of dsDNA-EC/LyoVec™ complex for 16 - 48 hours at 37°C, 5% CO₂.
- Monitor induction of type I IFNs by measuring the levels of IRF-induced SEAP in the cell culture supernatant using QUANTI-Blue™, a SEAP detection reagent.

Activation of NF-κB in HEK-Blue TLR9 cells

Activation of the TLR9-NF-κB pathway by intracellular dsDNA-EC can be monitored in HEK-Blue™ TLR9 cells.

- Prepare dsDNA-EC/LyoVec™ complex, as described above.
- Stimulate cells with 1 µg/ml dsDNA-EC/LyoVec™ complex for 16 - 24 hours at 37°C, 5% CO₂.
- Monitor activation of TLR9 by measuring the levels of NF-κB-induced SEAP in the cell culture supernatant using QUANTI-Blue™

RELATED PRODUCTS

Product	Catalog Code
LyoVec™	lyec-12
THP1-Blue™ ISG cells	thp-isg
HEK-Blue™ hTLR9 cells (human TLR9)	hkb-htlr9
HEK-Blue™ mTLR9 cells (mouse TLR9)	hkb-mtlr9
QUANTI-Blue™	rep-qbl
VACV-70 Naked	tlrl-vav70n
ISD Naked	tlrl-isdn
HSV-60 Naked	tlrl-hsv60n

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