ISD Naked

Bacterial DNA motif; CDS ligand

Catalog code: tlrl-isdn

https://www.invivogen.com/isd

For research use only

Version 24L09-MM

PRODUCT INFORMATION

Contents

- 200 µg ISD Naked
- 1.5 ml sterile endotoxin-free water

Sequence

5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTA-
3'-ATGTCTAGATGATCACTAGATACTGACTAGACATGTACTAGAT-

-CA-3'
-GT-5'

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- The ability of intracellular ISD to induce type I interferon (IFN) has been verified using cellular assays.
- The absence of bacterial contamination (e.g. lipoproteins and endotoxins) has been confirmed using HEK-Blue™ TLR2 and HEK-Blue™ TLR4 cells.

DESCRIPTION

Intracellular DNA from pathogens is recognized by multiple cytosolic DNA sensors (CDSs), which display contextual preferences for the recognition of DNA[1]. ISD (interferon stimulatory DNA) is a 45 bp non-CpG oligomer containing bacterial DNA motifs. ISD derives from the *Listeria monocytogenes* genome. When transfected into various cell types, including plasmacytoid and conventional DCs, macrophages, and murine embryonic fibroblasts, ISD strongly enhances the expression of IFN-β[2]. This ISD-induced response is mediated by the STING-TBK1-IRF3 signaling axis[2-3]. CDS ligands, including transfected ISD, trigger type I IFN production and the induction of interferon stimulated genes (ISG) through interferon regulatory factors (IRFs). In order to facilitate their study, InvivoGen has developed stable reporter cells in two well established immune cell models, the human monocytic THP-1 cell line and the murine RAW 264.7 macrophages. These cells express a reporter gene, either SEAP or Lucia® luciferase, a secreted luciferase, under the control of an IRF-inducible promoter. For more information visit www.invivogen.com/cell-lines.

1. Sharma S. & Fitzgerald KA. 2011. Innate immune sensing of DNA. PLoS Pathog. 7:e1001310. **2. Stetson DB & Medzhitov R. 2006.** Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. Immunity. 24:93-103. **3. Ishikawa H. *et al.*, 2009.** STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. Nature. 461:788-92. **4. Unterholzner L. *et al.*, 2010.** IFI16 is an innate immune sensor for intracellular DNA. Nat Immunol. 11:997-1004. **5. Zhang Z. *et al.*, 2011.** The helicase DDX41 senses intracellular DNA mediated by the adaptor STING in dendritic cells. Nat Immunol.12(10):959-65. **6. Arakawa R. *et al.*, 2010.** Characterization of LRRFIP1. Biochem Cell Biol. 88(6):899-906. **7. Lippmann J. *et al.*, 2010.** IFNbeta responses induced by intracellular bacteria or cytosolic DNA in different human cells do not require ZBP1 (DLM-1/DAI). Cell Microbiol. 10(12):2579-88.

Note: Lucia® is a registered trademark of InvivoGen.

METHODS

Preparation of stock solution (1 mg/ml)

1. Add 200 µl sterile endotoxin-free physiological water (provided) to 200 µg ISD Naked. Mix by pipetting up and down.

Preparation of ISD/cationic lipid complex

To facilitate the intracellular delivery, ISD Naked should be complexed with a cationic lipid transfection agent, such as LyoVec™. A protocol for the preparation of an ISD/LyoVec™ complex is given below:

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

Induction of type I IFNs

Induction of type I IFNs with ISD can be studied in a variety of cells including the human monocytic cell line THP-1. This cell line has been shown to express all the CDSs[4-6], with the exception of DAI[7]. A protocol for studying the induction of IFNs in THP1-Blue™ ISG cells is given below. These cells express an IFN regulatory factor (IRF)-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. Stimulation of this cell line with ISD/LyoVec™ activates the IFN pathway inducing SEAP production.

Note: The use of intracellular ISD Control as a negative control is highly recommended. This single-stranded oligonucleotide, unlike its double-stranded counterpart, does not induce type I IFNs.

1. Prepare ISD/cationic lipid complex as described above.
2. Stimulate THP1-Blue™ ISG cells with 100 ng/ml to 10 µg/ml of ISD/cationic lipid complex for 18-24 hours.
3. Monitor induction of type I IFNs by measuring the levels of SEAP in the cell culture supernatants using QUANTI-Blue™ Solution, a SEAP detection reagent.

RELATED PRODUCTS

Product	Catalog Code
LyoVec™	lyec-1
QUANTI-Blue™ Solution	rep-qbs
THP1-Blue™ ISG cells	thp-isg
ISD Control Naked	tlrl-isdcn

TECHNICAL SUPPORT
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