

ISD Control Naked

Bacterial DNA motif; Negative Control

Catalog code: tlrl-isdcn

<https://www.invivogen.com/isd-control>

For research use only

Version 24L10-MM

PRODUCT INFORMATION

Contents

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

Sequence

5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGATCTA-CA-3'

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 inability of intracellular ISD Control 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 DNA¹. ISD (interferon stimulatory DNA) Control is a negative control for the CDS agonist ISD. It is a non-immunostimulatory single-stranded oligonucleotide with the same sequence as ISD, its double-stranded counterpart. ISD is a 45-bp non-CpG oligomer from the *Listeria monocytogenes* genome. When transfected into various cell types, ISD strongly enhances the expression of IFN-β². 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. IFNβ 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.

TECHNICAL SUPPORT

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METHODS

Preparation of stock solution (1 mg/ml)

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

Preparation of ISD Control/cationic lipid complex

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

1. Rehydrate ISD Control Naked as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring ISD Control Naked and LyoVec™ to room temperature before use.
2. In a sterile 1.5 ml microfuge tube, mix 1 µg ISD Control 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.

Below is a protocol for determining type I IFN induction with a CDS ligand. ISD Control is a single-stranded oligonucleotide that does not induce type I IFNs. Use ISD Control/LyoVec™ at the same concentration as the double-stranded oligonucleotide ISD/LyoVec™.

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 DAI⁷. 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.

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 Naked	tlrl-isdcn