

ISD Naked

Bacterial DNA motif - CDS Ligand

Catalog # tlr1-isdn

For research use only

Version # 12L07-MM

PRODUCT INFORMATION

Content:

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

Sequence:

5'-TACAGTCTACTAGTGATCTATGACTGATCTGTACATGAT-
3'-ATGCTAGATGATCACTAGATACTGACTAGACATGTACTA-

-CTACA-3'

-GATGT-5'

Storage:

- ISD Naked is provided lyophilized and shipped at room temperature. Store lyophilized product at -20°C. Lyophilized product is stable for 12 months when properly stored.
- Upon resuspension, prepare aliquots and store ISD Naked at -20°C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

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) is a 45-bp non-CpG oligomer from the *Listeria monocytogenes* genome. When transfected into various cell types, including plasmacytoid and conventional dendritic cells (DCs), macrophages and murine embryonic fibroblasts, 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[®], a secreted luciferase, under the control of an IRF-inducible promoter.

InvivoGen provides ISD Control is a non-immunostimulatory single-stranded oligonucleotide with the same sequence as its double-stranded counterpart. For more information visit <http://www.invivogen.com/cds-ligands>

1. Sharma S. & Fitzgerald KA. 2011. Innate immune sensing of DNA. *PLoS Pathog.* 7(4):e1001310. 2. Stetson DB & Medzhitov R. 2006. Recognition of cytosolic DNA activates an IRF3-dependent innate immune response. *Immunity.* 24(1):93-103. 3. Ishikawa H. *et al.*, 2009. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature.* 461(7265):788-92. 4. Unterholzner L. *et al.*, 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(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*

METHODS

Preparation of stock solution (1 mg/ml)

Stimulation of CDS can be achieved with 100 ng - 10 µg/ml transfected ISD.

- Add 200 µl endotoxin-free water (provided) to 200 µg ISD Naked. Mix by pipetting up and down.
- Prepare aliquots and store at -20°C.

Preparation of ISD/cationic lipid complex

In order to facilitate the intracellular delivery of ISD, ISD should be complexed with a cationic lipid transfection agent, such as LyoVec[™]. A protocol for the extemporaneous preparation of a ISD/LyoVec[™] complex is given below:

- Rehydrate ISD as described above. Rehydrate LyoVec[™] as described on its technical data sheet. Bring ISD and LyoVec[™] to room temperature before use.

- In a sterile 1.5 ml microfuge tube, mix 1 µg ISD with 100 µl of LyoVec[™]. Homogenize gently.

- 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 in THP1-Lucia ISG cells

Induction of type I IFNs with ISD can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express all the CDSs^{2,4}, with the exception of DAI⁵. A protocol for the induction of type I IFNs using THP1-Lucia[™] ISG cells, an IRF-luciferase reporter cell line, is given below:

- Prepare ISD Naked/LyoVec[™] complex, as described above.

- Stimulate cells with 100 ng/ml - 10 µg/ml ISD/LyoVec[™] complex for 16 - 48 hours.

- Monitor induction of type I IFNs by measuring the levels of IRF-induced Lucia[®] in the cell culture supernatant using QUANTI-Luc[™], a Lucia[®] detection reagent.

RELATED PRODUCTS

Product	Catalog Code
LyoVec [™]	lyec-12
THP1-Lucia [™] ISG cells	thpl-isg
Raw-Lucia [™] ISG cells	rawl-isg
QUANTI-Luc [™]	rep-qlc1
ISD Control Naked	tlr1-isden
Other CDS ligands	
HSV-60 Naked	tlr1-hsv60n
pCpGfree-giant Naked	tlr1-cpgfn
VACV-70 Naked	tlr1-vav70n

TECHNICAL SUPPORT

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