

ISD/LyoVec™

Bacterial DNA motif complexed with LyoVec™ - CDS Ligand

Catalog # t1rl-isdc

For research use only

Version # 13D08-MM

PRODUCT INFORMATION

Content:

- 100 µg ISD/LyoVec™

Note: Each vial contains 25 µg of ISD complexed with 50 µg LyoVec™.

- 10 ml sterile endotoxin-free water

Sequence:

5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGAT-
3'-ATGTCTAGATGATCACTAGATACTGACTAGACATGTACTA-

-CTACA-3'

-GATGT-5'

Storage:

- ISD/LyoVec™ 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, store ISD/LyoVec™ at 4°C. Resuspended product is stable 1 week when properly stored.

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}. ISD is complexed with the cationic lipid LyoVec™ to facilitate its uptake.

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>

METHODS

Preparation of stock solution (50 µg/ml)

Stimulation of CDS can be achieved with 300 ng - 10 µg/ml ISD/LyoVec™.

- Add 500 µl sterile endotoxin-free water (provided) per vial of 25 µg ISD/LyoVec™. Mix gently. Allow at least 15 minutes for complete solubilization.

- Store at 4°C. Do not store for more than 1 week.

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^{4,6}, with the exception of DAI⁷. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:

- Resuspend ISD/LyoVec™, as described above.

- Stimulate cells with 300 ng/ml - 10 µg/ml ISD/LyoVec™ 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.

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 Microbiol. 10(12):2579-88.

RELATED PRODUCTS

Product	Catalog Code
THP1-Lucia™ ISG cells	thpl-isg
Raw-Lucia™ ISG cells	rawl-isg
QUANTI-Luc™	rep-q1c1
ISD Control/LyoVec™	t1rl-isdcc
Other CDS ligands	
HSV-60/LyoVec™	t1rl-hsv60c
pCpGfree-giant/LyoVec™	t1rl-cpgfc
VACV-70/LyoVec™	t1rl-vav70c

TECHNICAL SUPPORT

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