# ISD Control/LyoVec™

Bacterial DNA motif complexed with LyoVec™ - Negative Control

Catalog # tlrl-isdcc

For research use only

Version # 13D08-MM

## PRODUCT INFORMATION

**Content:**

- 100 µg ISD Control/LyoVec™

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

- 10 ml sterile endotoxin-free water

**Sequence:**

5'-TACAGATCTACTAGTGATCTATGACTGATCTGTACATGAT--CTACA-3'

**Storage:**

- ISD Control/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 Control/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 DNA[1]. 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-β[2]. This ISD-induced response is mediated by the STING-TBK1-IRF3 signaling axis[2-3].

ISD Control a non-immunostimulatory single-stranded oligonucleotide with the same sequence as its double-stranded counterpart. ISD Control 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. For more information visit http://www.invivogen.com/cds-cell-lines

## METHODS

**Preparation of stock solution (50 µg/ml)**

- Add 500 µl sterile endotoxin-free water (provided) per vial of 25 µg ISD Control/LyoVec™. Mix gently. Allow at least 15 minutes for complete solubilization.
- Store at 4°C. Do not store for more than 1 week.

Below is a protocol for determining type I IFN induction with a CDS ligand. Please note that 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 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 DAI[5]. 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 Control/LyoVec™, as described above.
- Stimulate cells with 300 ng/ml - 10 µg/ml ISD Control/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. Unterholzner L. *et al.*, 2010.** IFI16 is an innate immune sensor for intracellular DNA.Nat Immunol. 11(11):997-1004. **3. 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. **4. Arakawa R. *et al.*, 2010.** Characterization of LRRFIP1. Biochem Cell Biol. 88(6):899-906. **5. 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.

## RELATED PRODUCTS

| Product | Catalog Code |
|---|---|
| THP1-Lucia™ ISG cells | thpl-isg |
| Raw-Lucia™ ISG cells | rawl-isg |
| QUANTI-Luc™ | rep-qlc1 |
| **Other CDS ligands** | |
| ISD/LyoVec™ | tlrl-isdc |
| HSV-60/LyoVec™ | tlrl-hsv60c |
| pCpGfree-giant/LyoVec™ | tlrl-cpgfc |
| VACV-70/LyoVec™ | tlrl-vav70c |

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