

VACV-70c/LyoVec™

Viral DNA motif complexed with LyoVec™; Negative Control

Catalog code: tlrl-vav70cc

<https://www.invivogen.com/vacv70-control>

For research use only

Version 24L06-MM

PRODUCT INFORMATION

Contents

- 100 µg VACV-70c/LyoVec™

Note: Each vial contains 25 µg of VACV-70c complexed with 50 µg LyoVec™.

- 10 ml sterile endotoxin-free water

Sequence

5'-CCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGA-
-AGAGAGAAAGAGATAAACTTTTTTACGACT-3'

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, store at 4°C. Resuspended product is stable for 1 week when properly stored.

Quality control

- The inability of VACV-70c/LyoVec™ 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

VACV-70c (control) is a single-stranded 70 bp oligonucleotide. It is not an IFN-inducer, unlike its double-stranded counterpart, VACV-70. Intracellular DNA from pathogens is recognized by multiple cytosolic DNA sensors (CDSs), which display contextual preferences for the recognition of DNA¹. Transfected VACV-70 was shown to potentially induce interferon-beta (IFN-β) in a TLR-, DAI- and RNA Pol III-independent, but STING-, TBK1- and IRF3-dependent manner. VACV-70 is recognized by the CDSs, DDX41³ and IFI16¹. CDS ligands, including transfected VACV-70, 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. Unterholzner L. *et al.*, 2010. IFI16 is an innate immune sensor for intracellular DNA. *Nat Immunol.* 11(11):997-1004. 2. Sharma S. & Fitzgerald KA. 2011. Innate immune sensing of DNA. *PLoS Pathog.* 7(4):e1001310. 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.

Note: Lucia® is a registered trademark of InvivoGen.

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Asia: +852 3622-3480

E-mail: info@invivogen.com

METHODS

Preparation of stock solution (50 µg/ml)

1. Add 500 µl sterile endotoxin-free water (provided) per vial of 25 µg VACV-70c/LyoVec™. Mix gently. Allow at least 15 minutes for complete solubilization.
2. Store at 4°C. Do **not** store for more than 1 week.

Below is a protocol for the induction of type I IFN with a CDS ligand. Please note that VACV-70c is a single-stranded oligonucleotide that does not induce type I IFNs. Use VACV-70c/LyoVec™ at the same concentration as the double-stranded oligonucleotide VACV-70/LyoVec™.

Induction of type I IFNs

Induction of type I IFNs with VACV-70 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^{1,3,4}, with the exception of DAI⁵. 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.

1. Resuspend VACV-70c/LyoVec™, as described above.
2. Stimulate THP1-Blue™ ISG cells with 300 ng/ml to 10 µg/ml of VACV-70/LyoVec™ 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
QUANTI-Blue™ Solution	rep-qbs
THP1-Blue™ ISG cells	thp-isg
VACV-70c/LyoVec™	tlrl-vav70c