

VACV-70/LyoVec™

Viral DNA motif complexed with LyoVec™ - CDS Ligand

Catalog # tlr1-vav70c

For research use only

Version # 13D04-MM

PRODUCT INFORMATION

Content:

- 100 µg VACV-70/LyoVec™

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

- 10 ml endotoxin-free water

Sequence:

5'-CCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGA-

3'-GGTAGTCTTTCTCCAAATTATAAAAACACTCTGGTAGCT-

-AGAGAGAAAGAGATAAAAACACTTTTTTACGACT-3'

-TCTCTCTTTCTCTATTTTGAAAAATGCTGA-5'

Storage:

- VACV-70/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 VACV-70/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. VACV-70 is a double-stranded 70 bp oligonucleotide containing viral DNA motifs². VACV-70 derives from the vaccinia virus DNA. Transfected VACV-70 was shown to potently 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 IFI16². VACV-70 is complexed with the cationic lipid LyoVec™ to facilitate its uptake.

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®, a secreted luciferase, under the control of an IRF-inducible promoter.

InvivoGen provides VACV-70c (control) a single-stranded oligonucleotide complexed with LyoVec™, which unlike its double-stranded counterpart does not induce type I IFNs. 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 VACV-70/LyoVec™.

- Add 500 µl endotoxin-free water (provided) per vial of 25 µg VACV-70/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 VACV-70 can be studied in a variety of cells. The human monocytic cell line THP-1 has been shown to express all the CDSs²⁻⁴, 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:

- Resuspend VACV-70/LyoVec™, as described above.

- Stimulate cells with 300 ng/ml - 10 µg/ml VACV-70/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	thp1-isg
Raw-Lucia™ ISG cells	rawl-isg
QUANTI-Luc™	rep-qlc1
VACV-70c/LyoVec™ (control)	tlr1-vav70cc
Other CDS ligands	
ISD/LyoVec™	tlr1-isdc
pCpGfree-giant/LyoVec™	tlr1-cpgfc
HSV-60/LyoVec™	tlr1-hsv60c

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 Hong Kong: +852 3622-3480

E-mail: info@invivogen.com