VACV-70c/LyoVec™

Viral DNA motif complexed with LyoVec™ - Negative Control

Catalog # tlrl-vav70cc

For research use only

Version # 13D08-MM

PRODUCT INFORMATION

Content:

- 100 µg VACV-70c/LyoVec™

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

- 10 ml endotoxin-free water

Sequence:

5'-CCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGA--AGAGAGAAAGAGATAAAACTTTTTTACGACT-3'

Storage:

- VACV-70c/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-70c/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]. VACV-70c/LyoVec is a control for VACV-70/LyoVec. VACV-70c is complexed with the cationic lipid LyoVec™ to facilitate its uptake. VACV-70c is a single-stranded oligonucleotide, which unlike its double-stranded counterpart does not induce type I IFNs. VACV-70 derives from the vaccinia virus DNA[2]. Transfected double-stranded 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. Double-stranded VACV-70 is recognized by the CDSs, DDX41[3] and IFI16[2].

CDS ligands, including transfected double-stranded 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. For more information visit http://www.invivogen.com/cds-cell-lines

METHODS

Preparation of stock solution (50 µg/ml)

- Add 500 µl endotoxin-free water (provided) per vial of 25 µg VACV-70c/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 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 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 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 VACV-70c/LyoVec™, as described above.
- Stimulate cells with 300 ng/ml - 10 µg/ml VACV-70c/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
CDS ligands	
VACV-70/LyoVec™	tlrl-vav70c
ISD/LyoVec™	tlrl-isdc
HSV-60/LyoVec™	tlrl-hsv60c
pCpGfree-giant/LyoVec™	tlrl-cpgfc

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