

VACV-70c Naked

Viral DNA motif - Negative Control

Catalog # tlr1-vav70cn

For research use only

Version # 12L07-MM

PRODUCT INFORMATION

Content:

- 200 µg VACV-70c Naked
- 1.5 ml endotoxin-free water

Sequence:

5'-CCATCAGAAAGAGGGTTTAATATTTTTGTGAGACCATCGA-
-AGAGAGAAAGAGATAAACTTTTTTACGACT-3'

Storage:

- VACV-70c Naked 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, prepare aliquots and store VACV-70 Naked at -20°C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

DESCRIPTION

Intracellular DNA from pathogens is recognized by multiple cytosolic DNA sensors (CDSs), which display contextual preferences for the recognition of DNA¹. VACV-70c Naked is a control for VACV-70. VACV-70c Naked is a single-stranded oligonucleotide, which unlike its double-stranded counterpart does not induce type I IFNs. VACV-70 derives from the vaccinia virus DNA². 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. VACV-70 is recognized by the CDSs, DDX41³ and IFI16².

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. For more information visit <http://www.invivogen.com/cds-cell-lines>

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.

METHODS

Preparation of stock solution (1 mg/ml)

- Add 200 µl endotoxin-free water (provided) to 200 µg VACV-70c Naked. Mix by pipetting up and down.
- Prepare aliquots and store at -20°C.

Preparation of VACV-70c/cationic lipid complex

In order to facilitate the intracellular delivery of VACV-70c, VACV-70c should be complexed with a cationic lipid transfection agent, such as LyoVec™. A protocol for the extemporaneous preparation of a VACV-70c/LyoVec™ complex is given below:

- Rehydrate VACV-70c as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring VACV-70c and LyoVec™ to room temperature before use.
- In a sterile 1.5 ml microfuge tube, mix 1 µg VACV-70c with 100 µl of LyoVec™. Homogenize gently.
- Incubate at room temperature for 15 minutes to allow the formation of the complex. Do not store complex for more than 1 day.

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 DAI³. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-luciferase reporter cell line, is given below:

- Prepare VACV-70/LyoVec™ complex, as described above.
- Stimulate cells with 30 ng/ml - 10 µg/ml VACV-70/LyoVec™ complex 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.

RELATED PRODUCTS

Product	Catalog Code
LyoVec™	lyec-12
THP1-Lucia™ ISG cells	thp1-isg
Raw-Lucia™ ISG cells	rawl-isg
QUANTI-Luc™	rep-qlc1
Other CDS ligands	
VACV-70 Naked	tlr1-vav70n
ISD Naked	tlr1-isdn
HSV-60 Naked	tlr1-hsv60n

TECHNICAL SUPPORT

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