

# VACV-70 Naked

## Viral DNA motif - CDS Ligand

Catalog # ttrl-vav70n

For research use only

Version # 12L07-MM

### PRODUCT INFORMATION

#### Content:

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

#### Sequence:

5'-CCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGA-  
3'-GGTAGTCTTTCTCCAAATTATAAAAACTCTGGTAGCT-  
-AGAGAGAAAAGAGATAAAAACCTTTTTTACGACT-3'  
-TCTCTCTTTCTATTTTGA AAAAATGCTGA-5'

#### Storage:

- VACV-70 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 DNA<sup>1</sup>. VACV-70 is a double-stranded 70 bp oligonucleotide containing viral DNA motifs<sup>2</sup>. 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, DDX41<sup>3</sup> and IFI16<sup>2</sup>.

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<sup>®</sup>, a secreted luciferase, under the control of an IRF-inducible promoter.

InvivoGen provides VACV-70c Naked (control) a single-stranded oligonucleotide, which unlike its double-stranded counterpart does not induce type I IFNs. For more information visit <http://www.invivogen.com/cds-ligands>

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)

Stimulation of CDS can be achieved with 30 ng - 10 µg/ml transfected VACV-70.

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

#### Preparation of VACV-70/cationic lipid complex

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

- Rehydrate VACV-70 as described above. Rehydrate LyoVec<sup>™</sup> as described on its technical data sheet. Bring VACV-70 and LyoVec<sup>™</sup> to room temperature before use.

- In a sterile 1.5 ml microfuge tube, mix 1 µg VACV-70 with 100 µl of LyoVec<sup>™</sup>. 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.

#### 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<sup>2,4</sup>, with the exception of DAI<sup>5</sup>. A protocol for the induction of type I IFNs using THP1-Lucia<sup>™</sup> ISG cells, an IRF-luciferase reporter cell line, is given below:

- Prepare VACV-70/LyoVec<sup>™</sup> complex, as described above.

- Stimulate cells with 30 ng/ml - 10 µg/ml VACV-70/LyoVec<sup>™</sup> complex for 16 - 48 hours.

- Monitor induction of type I IFNs by measuring the levels of IRF-induced Lucia<sup>®</sup> in the cell culture supernatant using QUANTI-Luc<sup>™</sup>, a Lucia<sup>®</sup> detection reagent.

### RELATED PRODUCTS

Product	Catalog Code
LyoVec <sup>™</sup>	lyec-12
THP1-Lucia <sup>™</sup> ISG cells	thpl-isg
Raw-Lucia <sup>™</sup> ISG cells	rawl-isg
QUANTI-Luc <sup>™</sup>	rep-q1c1
VACV-70c Naked (control)	ttrl-vav70cn
<b>Other CDS ligands</b>	
ISD Naked	ttrl-isdn
pCpGfree-giant Naked	ttrl-cpgfn
HSV-60 Naked	ttrl-hsv60n

### TECHNICAL SUPPORT

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