VACV-70c Naked

Viral DNA motif; Negative Control

Catalog code: tlrl-vav70cn

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

For research use only

Version 24L06-MM

PRODUCT INFORMATION

Contents

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

Sequence

5'-CCATCAGAAAGAGGTTTAATATTTTTGTGAGACCATCGA--AGAGAGAAAGAGATAAAACTTTTTTACGACT-3'

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 6 months when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- The inability of intracellular VACV-70c 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 DNA[1]. VACV-70 contains viral DNA motifs derived from the vaccinia virus genome[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. VACV-70 is recognized by the CDSs, DDX41[3] and IFI16[2]. 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. 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)

1. Add 200 µl sterile endotoxin-free water (provided) to 200 µg VACV-70c Naked. Mix by pipetting up and down.

Preparation of VACV-70c/cationic lipid complex

To facilitate the intracellular delivery, VACV-70c Naked 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:

1. Rehydrate VACV-70c Naked as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring VACV-70c Naked and LyoVec™ to room temperature before use.
2. In a sterile 1.5 ml microfuge tube, mix 1 µg VACV-70c Naked with 100 µl of LyoVec™. Homogenize gently.
3. 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 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 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[2-4], with the exception of DAI[5]. 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. Prepare VACV-70c/cationic lipid complex as described above.
2. Stimulate THP1-Blue™ ISG cells with 30 ng/ml to 10 µg/ml of VACV-70c/cationic lipid complex 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
LyoVec™	lyec-1
QUANTI-Blue™ Solution	rep-qbs
THP1-Blue™ ISG cells	thp-isg
VACV-70 Naked	tlrl-vav70n

TECHNICAL SUPPORT
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