

HSV-60 Naked

Viral DNA motif; CDS Ligand

Catalog code: tlrl-hsv60n

<https://www.invivogen.com/hsv60>

For research use only

Version 21L23-MM

PRODUCT INFORMATION

Contents

- 200 µg HSV-60 Naked
- 1.5 ml endotoxin-free water

Sequence

5'-TAAGACACGATGCGATAAAATCTGTTTGTAAATTTATTA-
3'-ATTCTGTGCTACGCTATTTTAGACAAACATTTTAAATAAT-

-AGGGTACAAATTGCCCTAGC-3'

-TCCCATGTTTAACGGGATCG-5'

Storage and stability

- Product is shipped at room temperature. Upon receipt, store at -20°C.
- Upon resuspension, prepare aliquots of HSV-60 Naked and store at -20°C. Resuspended product is stable for 6 months at -20°C when properly stored. Avoid repeated freeze-thaw cycles.

Quality control

- The biological activity 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

Intracellular DNA from pathogens is recognized by multiple cytosolic DNA sensors (CDSs), which display contextual preferences for the recognition of DNA¹. HSV-60 is a double-stranded 60 bp oligonucleotide containing viral DNA motifs². HSV-60 derives from the herpes simplex virus 1 genome. Transfected HSV-60 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. HSV-60 is recognized by the CDSs, DDX41³ and IFI16².

CDS ligands, including transfected HSV-60, trigger type I IFN production and the induction of IFN stimulated genes (ISG) through IFN 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, secreted embryonic alkaline phosphatase or luciferase Lucia, under the control of an IRF-inducible promoter. For more information, visit <https://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.

TECHNICAL SUPPORT

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METHODS

Preparation of stock solution (1 mg/ml)

Stimulation of CDS can be achieved with 30 ng-10 µg/ml transfected HSV-60.

- Add 200 µl of endotoxin-free water (provided) to 200 µg of HSV-60 Naked. Mix by pipetting up and down.
- Prepare aliquots and store at -20°C.

Preparation of HSV-60/cationic lipid complex

In order to facilitate its intracellular delivery, HSV-60 should be complexed with a cationic lipid transfection agent, such as LyoVec™. A protocol for the extemporaneous preparation of a HSV-60/LyoVec™ complex is given below:

1. Rehydrate HSV-60 as described above. Rehydrate LyoVec™ as described on its technical data sheet. Bring HSV-60 and LyoVec™ to room temperature before use.
2. In a sterile 1.5 ml microfuge tube, mix 1 µg HSV-60 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.

Induction of type I IFNs in THP1-Lucia™ ISG cells

Induction of type I IFNs with HSV-60 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 DAI⁵. A protocol for the induction of type I IFNs using THP1-Lucia™ ISG cells, an IRF-Lucia luciferase reporter cell line, is given below:

1. Prepare HSV-60 Naked/LyoVec™ complex, as described above.
2. Stimulate cells with 30 ng/ml-10 µg/ml HSV-60/LyoVec™ complex for 16-48 hours.
3. Monitor induction of type I IFNs by measuring the levels of IRF-induced Lucia luciferase in the cell culture supernatant using QUANTI-Luc™, a Lucia luciferase detection reagent.

RELATED PRODUCTS

Product	Description	Cat. Code
ISD Naked	CDS ligand	tlrl-isdn
LyoVec™	Transfection reagent	lyec-1
QUANTI-Luc™	Lucia detection reagent	rep-qlc1
RAW-Lucia™ ISG Cells	Murine macrophages	rawl-isg
THP1-Lucia™ ISG Cells	Human monocytes	thpl-isg
VACV-70 Naked	CDS ligand	tlrl-vav70n