ssPoly(U) Naked

Single-stranded RNA polymer; Polyuridylic acid; human TLR8/mouse TLR7 agonist

Catalog code: tlrl-sspu

https://www.invivogen.com/sspolyu

For research use only

Version 19E21-MM

PRODUCT INFORMATION Contents:

• 10 mg of ssPoly(U) Naked

• 10 ml of endotoxin-free water

Storage and stability:

- ssPoly(U) Naked is provided lyophilized and shipped at room temperature. Store lyophilized product at -20°C.

- Upon resuspension, prepare aliquots of ssPoly(U) 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 validated 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

ssPoly(U) Naked (CAS number: 28086-43-3) is a synthetic ssRNA which can substitute for viral RNAs in inducing IFN- α production in plasmacytoid dendritic cells (PDC). Murine PDC deficient for TLR7 failed to produce IFN- α in response to poly(U), while the response to CpG-ODNs was unaffected, suggesting that TLR7 plays a critical role in viral ssRNA recognition¹. In human cells, TLR8 was shown to be the key receptor for viral ssRNA², implying a species specificity difference in ssRNA recognition. During infection, some viral particles are degraded by the endosomal proteases, exposing the viral genome and allowing TLR7 and/or TLR8 signaling, which are known to occur in endosomes³. TLR7 and TLR8 can recognize both self and viral RNA but seem able to distinguish the presence of viral RNA by detecting their abnormal localization in the endosome rather than a particular RNA motif.

1. Diebold SS. *et al.*, 2004. Innate antiviral responses by means of TLR7mediated recognition of single-stranded RNA. Science. 5;303(5663):1529-31 2. Heil F. *et al.*, 2004. Species-specific recognition of single-stranded RNA via toll-like receptor 7 and 8. Science. 5;303(5663):1526-9. 3. Heil F. *et al.*, 2003. The Toll-like receptor 7 (TLR7)-specific stimulus loxoribine uncover a strong relationship within the TLR7, 8 and 9 subfamily. Eur J Immunol. 33(11):2987-97.

METHODS

Preparation of ssPoly(U) Naked stock solution (1 mg/ml)

- Add 10 ml of sterile endotoxin-free water to 10 mg of ssPoly(U) Naked. Mix gently until completely dissolved.

Human TLR8 / Mouse TLR7 stimulation

ssPolyU can be used to stimulate TLR7 or TLR8 in HEK-Blue[™] TLR7 or TLR8 cells. These cells stably express an NF-kB-inducible secreted embryonic alkaline phosphatase (SEAP) and overexpress the TLR7 or the TLR8 gene.

For more information visit: <u>https://www.invivogen.com/hek-blue-tlr</u>

1. Rehydrate ssPoly(U) Naked and LyoVec[™] at the recommended concentrations. Bring ssPoly(U) Naked and LyoVec[™] to room temperature and gently vortex to homogenize before use.

2. In a sterile 1.5 ml microfuge tube at room temperature, mix 1 µl (1 µg) ssPoly(U) Naked stock solution (1 mg/ml) with 100 µl of LyoVec[™]. Mix gently.

3. Incubate at room temperature for 15 minutes to allow the formation of the complex.

4. Add 10-20 µl of ssPoly(U) & LyoVec[™] complex to each well of a 96-well plate.

5. To each well containing ssPoly(U) & LyoVec[™], add 180 µl of a HEK-Blue[™] hTLR8 or HEK-Blue[™] hTLR8 cell suspension.

6. Incubate for 6-24 h at 37 °C, 5% CO₂.

7. Determine TLR stimulation using a SEAP detection medium, such as QUANTI-Blue[™] Solution or HEK-Blue[™] Detection.

RELATED PRODUCTS

Product	Description	Cat.Code
CL264	TLR7 ligand	tlrl-c264s
Gardiquimod [™]	TLR7 ligand	tlrl-gdqs
HEK-Blue [™] mTLR7 cells	Murine TLR7 reporter cells	hkb-mtlr7
HEK-Blue [™] hTLR8 cells	Human TLR8 reporter cells	hkb-htlr8
HEK-Blue [™] Detection	SEAP detection reagent	hb-det2
Imiquimod (R837)	TLR7 ligand	tlrl-imqs
ORN06/LyoVec [™]	TLR8 ligand	tlrl-orn6
QUANTI-Blue [™] Solution	SEAP detection reagent	rep-qbs
ssPolyU/LyoVec [™]	TLR7/TLR8 ligand	tlrl-lpu
ssRNA40/LyoVec [™]	TLR8 ligand	tlrl-lrna40

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