

Poly(U)

Single-stranded RNA polymer - Polyuridylic acid

Catalog # tlr1-sspu, tlr1-sspu-100

For research use only

Version # 10K05-MM

PRODUCT INFORMATION

Contents:

Poly(U) is provided lyophilized and is available in two sizes:

- 10 mg: tlr1-sspu
- 100 mg (10 x10 mg): tlr1-sspu-100
- 10 ml endotoxin-free water is provided with tlr1-sspu and 100 ml (4 x 25 ml) endotoxin-free water is provided with tlr1-sspu-100.

Storage and stability:

- Poly(U) is provided lyophilized and shipped at room temperature. Store lyophilized Poly(U) at -20°C. Lyophilized product is stable 1 year when stored -20°C.
- Upon resuspension, store Poly(U) at 4°C. Resuspended product is stable for 1 week at 4 °C.

Quality control:

- λ max in H₂O: 260 +/- 5 nm
- 280/260 in 0.9 % w/v NaCl: 0.35 +/- 0.05
- Endotoxin level: <5 EU/mg
- Activity tested using HEK-Blue™ hTLR8 cells

DESCRIPTION

Poly(U) (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 TLR7-mediated 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 uncovers a strong relationship within the TLR7, 8 and 9 subfamily. *Eur J Immunol*. 33(11):2987-97.

METHODS

Preparation of Poly(U) stock solution (1 mg/ml)

- Add 10 ml of sterile endotoxin-free water to the 10 mg of Poly(U). Mix gently until complete solubilization.

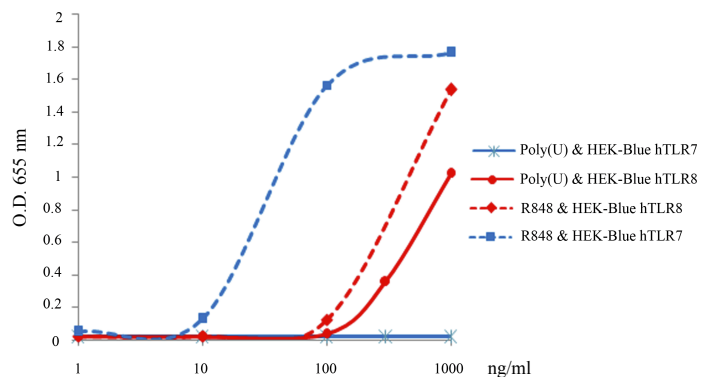
Stimulation of mouse TLR7 or human TLR8

Stimulation of mouse TLR7 (mTLR7) and human TLR8 (hTLR8) can be achieved with poly(U) in cells expressing human TLR8 or cells expressing the mouse TLR7. To achieve poly(U) stimulation of hTLR8 or mTLR7, the poly(U) must be delivered intracellularly, for example by using a transfection agent, such as LyoVec™.

Note: If your cell line does not naturally express the mouse TLR7 or human TLR8 gene, transfect with a plasmid expressing either TLR gene, such as pUNO-mTLR7 or pUNO1-hTLR8b. Cells can also be transfected with an NF- κ B-inducible reporter plasmid to quantify the stimulation of hTLR8 or mTLR7. InvivoGen provides pNiFty, a family of NF- κ B-inducible reporter plasmids that can be transfected transiently (pNiFty) or stably (pNiFty2). pNiFty plasmids are available either with the SEAP or luciferase reporter genes.

Example of hTLR8 stimulation using Poly(U) and LyoVec™ transfection reagent in HEK-Blue™ hTLR8 Cells

Poly(U) & LyoVec™ can be used to stimulate hTLR8 in HEK-Blue™ hTLR8 cells. HEK-Blue™-hTLR8 cells are designed for studying the stimulation of hTLR8 by monitoring the activation of NF- κ B. Stimulation with a TLR8 ligand activates NF- κ B and AP-1 which induces the production of SEAP. Levels of SEAP can be easily determined with QUANTI-Blue™ (a detection medium that turns blue in the presence of alkaline phosphatase). A typical stimulation curve is given below.



Human TLR8 specificity of Poly(U) stimulation. HEK-Blue™ hTLR8 cells respond to stimulation with Poly(U) & LyoVec™, while HEK-Blue™ hTLR7 cells do not respond to stimulation with Poly(U) & LyoVec™. Both HEK-Blue™ hTLR8 and HEK-Blue™ hTLR7 cells respond to stimulation with R848. Levels of SEAP were determined with QUANTI-Blue™.

TECHNICAL SUPPORT

Toll free (US): 888-457-5873
Outside US: (+1) 858-457-5873
Europe: +33 562-71-69-39
E-mail: info@invivogen.com
Website: www.invivogen.com


3950 Sorrento Valley Blvd. Suite 100
San Diego, CA 92121 - USA

Protocol

- Rehydrate Poly(U) and LyoVec™ at the recommended concentrations. Bring LyoVec™ and Poly(U) to room temperature and gently vortex to homogenize before use.
- In a sterile 1.5 ml microfuge tube at room temperature, mix 1 µl (1 µg) Poly(U) stock solution (1 mg/ml) with 100 µl of LyoVec™. Mix gently.
- Incubate at room temperature for 15 minutes to allow the formation of the complex.
- Add 10 - 20 µl of Poly(U) & LyoVec™ complex to each well of a 96-well plate.
- To each well containing Poly(U) & LyoVec™, add 180 µl of a HEK-Blue™ hTLR8 cell suspension (200,000 cells/ml) in DMEM, 4.5 g/l glucose, 10% (v/v) heat-inactivated fetal bovine serum (30 min at 56°C), 50 U/ml penicillin, 50 µg/ml streptomycin, 100 µg/ml Normocin™, 2 mM L-glutamine .
- Incubate for 24 hours at 37°C.
- Determine Poly(U) stimulation on human TLR8 by SEAP reporter gene expression using QUANTI-Blue™ or HEK-Blue™ detection .

RELATED PRODUCTS

Product	Catalog Code
HEK-Blue™ hTLR8 Cells	hkb-htlr8
pNiFty2-Luc (Zeo ^R)	pnifty2-luc
pNiFty2-SEAP (Zeo ^R)	pnifty2-seap
pUNO-mTLR7 (mouse gene)	puno-mtlr7
pUNO1-hTLR8b (human gene)	puno1-htlr8b
293XL/mTLR7 (mouse gene)	293xl-mtlr7
293XL/hTLR8A (human gene)	293xl-htlr8
ssPoly(U)/LyoVec™	tlrl-lpu
LyoVec™	lyvec-12
QUANTI-Blue™ (5 pouches)	rep-qb1
HEK-Blue™ Detection (2 pouches)	hb-det1
Normocin™	ant-nr-1

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Europe: +33 562-71-69-39
E-mail: info@invivogen.com
Website: www.invivogen.com

 **InvivoGen**
3950 Sorrento Valley Blvd. Suite 100
San Diego, CA 92121 - USA