

LFn-Needle

T3SS Needle protein fused to Lethal Factor; NLRC4 inflammasome inducer
Catalog code: tlrl-ndl

<https://www.invivogen.com/lfn-needle>

For research use only

Version 21A11-NJ

PRODUCT INFORMATION

Contents

- 5 µg of lyophilized LFn-Needle protein
- 1.5 ml endotoxin-free water

Note: B. anthracis protective antigen (PA) is not provided.

Protein construction

T3SS Needle protein [S2-R89] fused to the amino-terminal domain [A34-R296] of anthrax toxin's lethal factor (LFn) protein in N-terminal.

Accession sequence: WP_009896110 (Needle sequence)

Species: *Burkholderia thailandensis*

Source: Sf9 insect cells

Tag: N-terminal poly-histidine (6 x His)

Total protein size: 371 a.a. (secreted form)

Molecular weight: ~ 46 KDa (SDS-PAGE)

Purification: Ni²⁺ affinity chromatography

Purity: >90% (SDS-PAGE)

Formulation: Lyophilized from 0.2 µm filtered solution in 150 mM sodium chloride, 20 mM sodium phosphate buffer with 2% human serum albumin (HSA) and 5% saccharose

Storage and stability

- LFn-Needle is shipped at room temperature.
 - Upon receipt, store LFn-Needle at -20°C for up to 6 months.
 - Upon resuspension, store aliquots at -20°C for up to 6 months.
- Avoid repeated freeze-thaw cycles.

Quality control

- Size and purity of the protein have been confirmed by SDS-PAGE.
- 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.

PRODUCT DESCRIPTION

LFn-Needle is a model of NLRC4/NAIP inflammasome agonist^{1,2}. Needle is a component of the type III secretion systems (T3SS) of intracellular bacteria described as an NLRC4/NAIP ligand^{1,3}. It is fused to the amino-terminal domain of *B. anthracis* lethal factor (LFn). This fusion system, when co-administered with the anthrax toxin's protective antigen (PA), allows intracellular delivery of the bacterial ligand⁴. The combination of LFn-Needle with the anthrax protective antigen (PA) is named Needle-Tox².

1. Zhao Y., et al. 2011. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature*. 477(7366):596-600. **2. Rauch I. et al., 2016.** NAIP proteins are required for cytosolic detection of specific bacterial ligands in vivo. *The Journal of Exp. Med.* 213(5):657-665. **3. Worrall L.J. et al., 2011.** Structural overview of the bacterial injectisome. *Curr Opin Microbiology*. 14(1):3-8. **4. Ballard J.D. et al., 1996.** Anthrax toxin-mediated delivery of a cytotoxic T-cell epitope in vivo. *PNAS*. 93(22):12531-12534.

METHODS

LFn-Needle resuspension (100 µg/ml)

Note: Ensure you see the lyophilized pellet before resuspension.

- Add 50 µl of endotoxin-free water to the vial and gently pipette until completely resuspended.
- Prepare aliquots and store at -20 °C.

Working concentrations: 0.16 - 100 ng/ml (final concentration)

Note: LFn-Needle used in combination with the B. anthracis Protective antigen (PA) allows its translocation into the cytosol. The combination is sometimes referred to as Needle-Tox².

NLRC4 INFLAMMASOME INDUCTION

LFn-Needle can be used to induce the NLRC4 inflammasome in cellular assays, such as InvivoGen's THP1-NLRC4, THP1-KO-NLRC4, and THP1-Null2 cell lines. The production IL-1β by these cells is measured using HEK-Blue™ IL-1β cells.

Production of IL-1β by THP1-derived cells

1. If using InvivoGen's cell lines, please refer to their technical data sheet for information regarding growth and test conditions.
2. Prepare test medium: RPMI 1640, 2 mM L-glutamine, 25 mM HEPES, 10% (v/v) heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin.
3. Prepare a 1.6 x 10⁶ cells/ml suspension in test medium and add 180 µl of cell suspension per well of a 96-well plate (~3 x 10⁵ cells/well).
4. Prime cells with 20 µl of LPS-EK (final concentration 1 µg/ml) for 3 hours at 37 °C in 5% CO₂.
5. Carefully remove medium and add 180 µl test medium.
6. Add 10 µl of *B. anthracis* Protective antigen (PA) (final concentration 20 ng/ml).
- Note: The concentration of PA remains the same in each well.*
7. Add 10 µl of LFn-Needle (final concentration 100 - 0.16 ng/ml).
8. Incubate overnight at 37 °C in 5% CO₂.

Detection of IL-1β

Secreted IL-1β from the supernatant of the treated THP-1 cells can be detected using InvivoGen's HEK-Blue™ IL-1β cells. For more information, visit <https://www.invivogen.com/hek-blue-il1b>.

RELATED PRODUCTS

Product	Description	Cat. Code
LPS-EK (<i>E. coli</i> K12)	TLR4 agonist	tlrl-pek1ps
HEK-Blue™ IL-1β cells	IL-1β reporter cells	hkb-il1bv2
QUANTI-Blue™ Solution	SEAP detection reagent	rep-qbs1
THP1-Null2 Cells	Human monocytic cells	thp-null2
THP1-NLRC4	Inflammasome test cells	thp-nlrc4
THP1-KO-NLRC4	Inflammasome test cells	thp-konlrc4

TECHNICAL SUPPORT

InvivoGen USA (Toll-Free): 888-457-5873

InvivoGen USA (International): +1 (858) 457-5873

InvivoGen Europe: +33 (0) 5-62-71-69-39

InvivoGen Hong Kong: +852 3622-3480

E-mail: info@invivogen.com

