

# LFn-Rod

T3SS Inner Rod protein fused to Lethal Factor; NLRC4 inflammasome inducer  
Catalog code: tlr1-rod

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

For research use only

Version 21A11-NJ

## PRODUCT INFORMATION

### Contents

- 50 µg of lyophilized LFn-Rod protein
- 1.5 ml endotoxin-free water

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

### Protein construction

T3SS Inner Rod protein [M1-S101] fused to the amino-terminal domain [A34-R296] of anthrax toxin's lethal factor (LFn) protein in N-terminal.

**Accession sequence:** WP\_000020431 (Inner Rod; PrgJ sequence)

**Species:** *Salmonella typhimurium*

**Source:** Sf9 insect cells

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

**Total protein size:** 384 a.a. (secreted form)

**Molecular weight:** ~ 48 KDa (SDS-PAGE)

**Purification:** Ni<sup>2+</sup> 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-Rod is shipped at room temperature.
  - Upon receipt, store LFn-Rod 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-Rod is a model of NLRC4/NAIP inflammasome agonist<sup>1-3</sup>. Inner Rod is a component of the type III secretion systems (T3SS) of intracellular bacteria described as an NLRC4/NAIP ligand<sup>1-4</sup>. 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 ligand<sup>5</sup>. The combination of LFn-Rod with the anthrax protective antigen (PA) is named Rod-Tox<sup>3</sup>.

1. Zhao Y. et al., 2011. The NLRC4 inflammasome receptors for bacterial flagellin and type III secretion apparatus. *Nature*. 477(7366):596-600. 2. Kofoed E.M. & Vance R.E., 2011. Innate immune recognition of bacterial ligands by NAIPs determines inflammasome specificity. *Nature*. 477(7366):592-595. 3. 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. 4. Worrall L.J. et al., 2011. Structural overview of the bacterial injectisome. *Curr Opin Microbiology*. 14(1):3-8. 5. 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-Rod resuspension (1.9 mg/ml)

*Note:* Ensure you see the lyophilized pellet before resuspension.

- Add 26.3 µl of endotoxin-free water to the vial and gently pipette until completely resuspended.
- Prepare aliquots and store at -20 °C.

**Working concentrations:** 16 ng/ml - 10 µg/ml (final concentration)

*Note:* LFn-Rod used in combination with the *B. anthracis* Protective antigen (PA) allows its translocation into the cytosol. The combination is sometimes referred to as Rod-Tox<sup>3</sup>.

## NLRC4 INFLAMMASOME INDUCTION

LFn-Rod can be used to induce the NLRC4 inflammasome in cellular assays, such as InvivoGen's RAW-ASC and RAW-ASC KO-NLRC4 cell lines.

### Production of IL-1β by RAW-ASC-derived cells

1. Please refer to InvivoGen's technical data sheets for information regarding growth and test conditions of our cell lines.
2. Prepare test medium: DMEM without phenol red, 4.5 g/l glucose, 4 mM L-glutamine, 10% heat-inactivated FBS, 100 U/ml penicillin, 100 µg/ml streptomycin.
3. Prepare a 1.1 x 10<sup>6</sup> cells/ml suspension in test medium and add 180 µl of cell suspension per well of a 96-well plate (~2 x 10<sup>5</sup> cells/well).
4. Prime cells with 20 µl of Pam3CSK4 (final concentration 100 ng/ml) for 3 hours at 37 °C in 5% CO<sub>2</sub>.
5. Carefully remove medium and add 180 µl test medium.
6. Add 10 µl of *B. anthracis* Protective antigen (PA) (final concentration 1 µg/ml).
- Note:* The concentration of PA remains the same in each well.
7. Add 10 µl of LFn-Rod to the cells (final concentration 10 µg/ml - 16 ng/ml).
8. Incubate overnight at 37 °C in 5% CO<sub>2</sub>.

## RELATED PRODUCTS

Product	Description	Cat. Code
Pam3CSK4	TLR1/2 agonist	tlr1-pms
RAW-ASC cells	Inflammasome test cells	raw-asc
RAW-ASC KO-NLRC4 cells	Inflammasome test cells	raw-asc-ko-nlrc4

## TECHNICAL SUPPORT

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