

# Necrostatin-1

Inhibitor of RIPK1 (receptor-interacting protein kinase 1) - InvitroFit™

Catalog code: inh-ncst1, inh-ncst1-4

<https://www.invivogen.com/necrostatin-1>

For research use only

Version 23L08-MM

## PRODUCT INFORMATION

Contents Necrostatin-1 is available in two quantities:

- **inh-ncst1:** 25 mg Necrostatin-1 - InvitroFit™
- **inh-ncst1-4:** 4 x 25 mg Necrostatin-1 - InvitroFit™

### Storage and stability

- Necrostatin-1 is provided as a powder and shipped at room temperature. Upon receipt, store at -20 °C.
- Upon resuspension, prepare aliquots and store at -20 °C. Resuspended product is stable for 6 months at -20 °C when properly stored. Avoid repeated freeze-thaw cycles.

*Note:* We recommend to protect this product from light.

### Quality control

- Purity: ≥95% (UHPLC)
- The inhibitory activity has been confirmed 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

Necrostatin-1 (Nec-1) is a potent and specific small-molecule inhibitor of the receptor-interacting serine/threonine-protein kinase 1 (RIPK1, aka RIP1)<sup>1,2</sup>. RIPK1, through its kinase and scaffolding functions, is a key regulator of apoptosis, necroptosis and inflammatory pathways. The Ser/Thr kinase activity of RIPK1 is essential for RIPK1-RIPK3-MLKL necrosome-mediated necroptosis upon TNF- $\alpha$  (tumor necrosis factor- $\alpha$ ) signaling<sup>2,3</sup>. Necrostatin-1 binds within the hydrophobic pocket of RIPK1, located between the N- and C-terminus of the kinase domain, and locks RIPK1 in an inactive conformation<sup>4</sup>. The administration of necrostatin-1 reduces cell death and/or mortality in several disease models<sup>3</sup>.

1. Degtarev A. *et al.*, 2005. Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. *Nat. Chem. Biol.* 1: 112-119.
2. Degtarev A. *et al.*, 2008. Identification of RIP1 kinase as a specific cellular target of necrostatins. *Nature Chemical Biology* 4(5), 313-321.
3. Speir M. *et al.*, 2021. Targeting RIP kinases in chronic inflammatory disease. *Biomolecules*. 11:646.
4. Xie T. *et al.*, 2013. Structural Basis of RIP1 Inhibition by Necrostatins. *Structure*. 21(3):493-499.

## CHEMICAL PROPERTIES

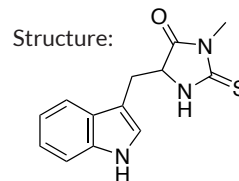
CAS number: 4311-88-0

Synonym: 5-(1H-Indol-3-ylmethyl)-3-methyl-2-thioxo-4-imidazolidinone

Formula: C<sub>13</sub>H<sub>13</sub>N<sub>3</sub>OS

Molecular weight: 259.33 g/mol

Solubility: 10 mg/ml (38.6 mM) in DMSO



## METHODS

### Preparation of stock solution (38.6 mM)

1. Add 2.5 ml DMSO to 25 mg Necrostatin-1 vial.
2. Vortex until completely resuspended.
3. Prepare aliquots of Necrostatin-1 and store at -20 °C.
4. Once Necrostatin-1 is resuspended, further dilutions can be prepared using sterile aqueous buffers.

Working concentration range: 0.15 - 40  $\mu$ M for cell culture assays

## PROTOCOL

Below is a protocol for measuring cell death using THP1-HMGB1-Lucia™ cells. This assay relies on the luminescence quantification of the HMGB1::Lucia fusion protein released in the supernatant upon pyroptosis or necroptosis. For more information, visit: <https://www.invivogen.com/thp1-hmgb1-lucia>.

### Necroptosis assay

It is recommended to perform the assay with test medium which does not contain Normocin™ nor Zeocin™.

1. Add 20  $\mu$ l of a caspase inhibitor such as Z-VAD-FMK (25  $\mu$ M final concentration) per well of a flat-bottom 96-well plate.
2. Prepare a THP1-HMGB1-Lucia™ suspension at  $\sim 2.5 \times 10^6$  cells/ml.
3. Dispense 120  $\mu$ l of cell suspension ( $\sim 300,000$  cells) per well.
4. Incubate at 37 °C in 5% CO<sub>2</sub> for 1 h.
5. Add 20  $\mu$ l of a cIAP inhibitor such as BV6 (5  $\mu$ M final concentration), 20  $\mu$ l of recombinant hTNF- $\alpha$  (100 ng/ml final concentration), and 20  $\mu$ l of RIPK1 inhibitor (Necrostatin-1, 40  $\mu$ M final concentration) per well.
6. Incubate the plate at 37 °C in a CO<sub>2</sub> incubator for 8-24 h. Proceed to detection of HMGB1::Lucia using QUANTI-Luc™ 4 Lucia/Gaussia as described on the next page.

## TECHNICAL SUPPORT

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### Detection of HMGB1::Lucia

Below is a protocol for end-point readings using a luminometer. This protocol can be adapted for use with kinetic measurements.

1. Prepare the [QUANTI-Luc™ 4 Lucia/Gaussia](#) assay solution following the instructions on the enclosed data sheet.
2. Transfer 10 µl of THP1-HMGB1-Lucia™ stimulated cell supernatant into a 96-well white (opaque) or black plate, or a luminometer tube.
3. Add 50 µl of [QUANTI-Luc™ 4 Lucia/Gaussia](#).
4. Proceed **immediately** with the measurement.

## RELATED PRODUCTS

Product	Description	Cat. Code
BV6	IAP inhibitor	inh-bv6
Z-IETD-FMK	Caspase-8 inhibitor	inh-ietd
Z-VAD-FMK	Pan-caspase inhibitor	tlrl-vad
Recombinant hTNF- $\alpha$	Recombinant cytokine	rcyc-htrnf $\alpha$
THP1-HMGB1-Lucia™	Reporter cell line	thp-gb1lc
QUANTI-Luc™ 4 Lucia/Gaussia	Detection reagent	rep-qlc4lg1

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