Nigericin

CATALOG #6698
FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Activation of the NLRP3 inflammasome in myeloid cells follows exposure to pathogen-associated molecular patterns (first signal) and ATP (second signal). This leads to oligomerization and assembly of a high molecular weight (~700 kDa) multimeric inflammasome complex, which leads to the conversion of pro-caspase-1 into the catalytically active form. Inflammatory caspases, such as caspase-1, or interleukin-converting enzyme, play a central role in innate immunity by recognizing cytosolic foreign danger signals and initiating a two-fold response. First, caspase-1, proteolytically converts the proforms of the two important pro-inflammatory cytokines, interleukin 1ß (IL-1ß) and interleukin 18 (IL-18), into their active forms, which are secreted. Second, caspase-1 or caspase-11 triggers a form of lytic, programmed cell death known as pyroptosis

Nigericin is a potent microbial toxin derived from *Streptomyces hygroscopicus*. It acts as a potassium ionophore, inducing a net decrease in intracellular levels of potassium, which is crucial for oligomerization of the NLRP3 inflammasome and activation of caspase-1. Nigericin requires signaling through pannexin-1 to induce caspase-1 activation and IL-1ß processing and release. It has been shown to generate a robust caspase-1 activation response in various cell lines, including Jurkat and THP-I cells.

WARNING

- Danger! Nigericin is toxic if swallowed, causes skin irritation, causes serious eye irritation, and may cause respiratory irritation.
- SDS available at www.immunochemistry.com and by calling ICT at 952-888-8788 or 800-829-3194.
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SPECIFICATIONS

- 0.5 μmoles per vial
- MW: 746.94 g/mol
- CAS# 28643-80-3
- Store: ≤ -20°C
- Upon reconstitution in DMSO, store at -20°C.
- Reconstituted Nigericin is stable for up to 1 year at -20°C.

HOW TO USE

Nigericin is supplied lyophilized at 0.5 μmoles per vial. It may be slightly visible as an iridescent sheen or white powder inside the vial. Once reconstituted in DMSO, Nigericin is ready to use. Protect from light and use gloves when handling.

1. Reconstitute each vial of Nigericin with 100 μL DMSO to form the 5 mM stock concentrate. Once reconstituted, it may be aliquoted and stored at ≤-20°C for 1 year protected from light and thawed no more than twice during that time.
2. Immediately prior to addition to the samples and controls, dilute 5 mM Nigericin stock 1:10 in diH_2O to form a 500 μM working solution for use in treating samples. For example, dilute 1:10 by adding 20 μL stock concentrate to 180 μL diH_2O.
3. Use Nigericin at 1-20 μM to induce NLRP3 inflammasome activation in cells. For example, to use at 10 μM, dilute 500 μM working solution 1:50 in samples; e.g., spike 294 μL cell suspension/overlay medium with 6 μL of 500 μM working solution. Typical treatment periods range from 3-24 hours at 37°C. Each investigator should adjust the concentration of Nigericin and treatment period to accommodate the particular cell line and research conditions.
4. NLRP3 inflammasome activation can be detected by ELISA or Western blot measuring secreted pro-inflammatory cytokines IL-1ß or IL-18, or through the use of caspase-1 activation assays, such as ICT's Caspase-1 Assay Kits (#97, 98, 9122) or ICT's new Pyroptosis/Caspase-1 Assay Kits (#9145, 9146). See Figure 1 for time-course study in Jurkat cells treated with 10 μM Nigericin for a period of 1-24 hours at 37°C.

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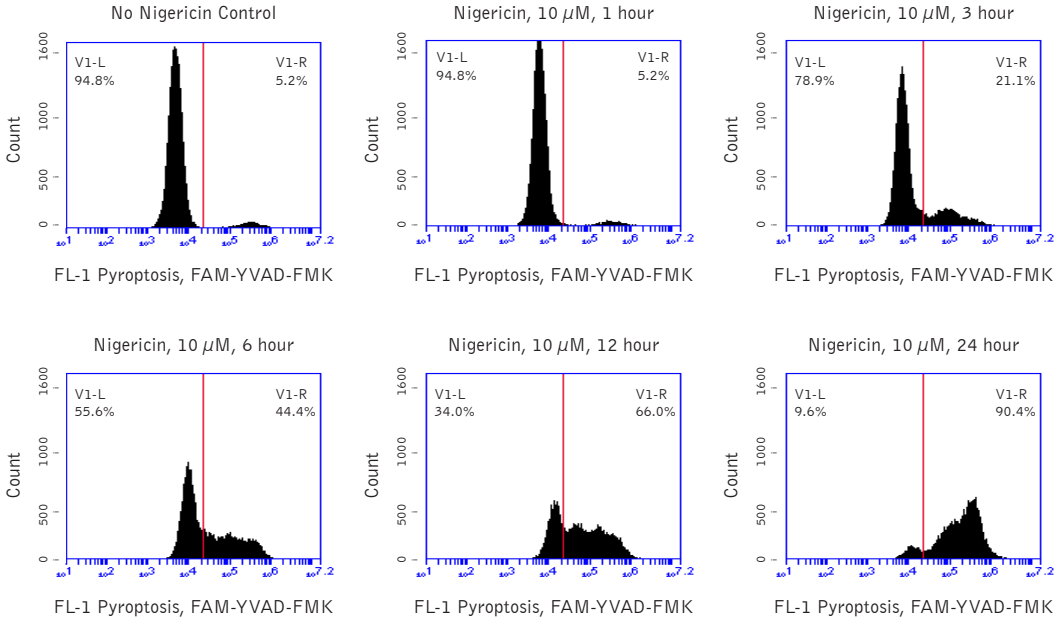
Figure 1: Time-Course Response in Jurkat Cells to Nigericin-Induced Caspase-1 Activation

ICT's caspase-1 inhibitor reagent, FAM-YVAD-FMK (kit catalog #9146) was used to monitor the caspase-1 induction response in Jurkat cells treated with Nigericin for various periods of time. A common cell pool was spiked with FAM-YVAD-FMK and divided into separate treatment groups. Starting with 24 hour samples and working backwards, 10 μM Nigericin was added to cells and the samples were incubated at 37°C throughout the induction process. Following their respective treatment exposure periods, the cells were washed and analyzed on an Accuri C6 flow cytometer. The amount of caspase-1 activity detected directly correlated to the duration of the exposure period; the longer the cells were exposed to Nigericin, the larger the proportion of caspase-1 positive cells found in the sample. Data courtesy of Mrs. Tracy Murphy, ICT (220:78).



Thank you for using Nigericin! If you have any questions, or would like to share your data, please contact us at help@immunochemistry.com.

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