

# Validation data for Z-VAD-FMK

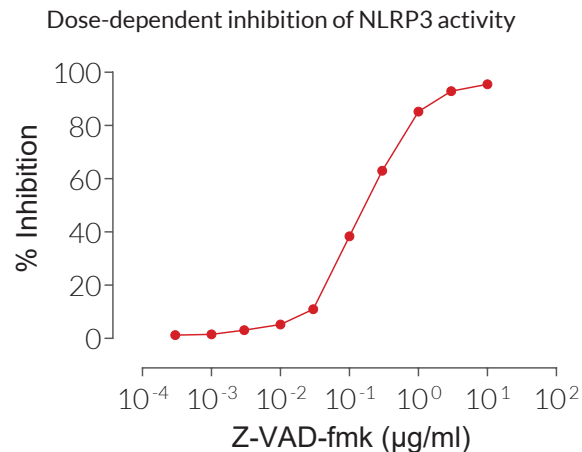
<https://www.invivogen.com/z-vad-fmk>

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Z-VAD-FMK is a broad spectrum inhibitor of caspases, cytosolic proteases involved in the regulation of inflammation and cell death. Of note, this pan-caspase inhibitor is often used to block caspase-1 activity in inflammasome activation studies. The inflammasome is an innate immune sensor that is activated by a two-step process; a first signal ('priming') is provided by microbial molecules such as lipopolysaccharide (LPS), while the second signal is provided by a wide array of stimuli including endogenous molecules, such as double-stranded DNA (dsDNA), or crystalline substances such as monosodium urate (MSU) crystals. Inflammasome activation triggers caspase-1-mediated interleukin-1 $\beta$  (IL-1 $\beta$ ) production and secretion.

The ability of Z-VAD-FMK to inhibit the NLRP3 (NOD-like receptor (NLR) pyrin domain-containing protein 3) inflammasome was validated using InvivoGen's THP-1/HEK-Blue™ IL-1 $\beta$  assay. This assay uses the secretion of IL-1 $\beta$  by THP1-Null2 cells as an indicator of NLRP3 inflammasome induction. The IL-1 $\beta$  production by these cells is measured using HEK-Blue™ IL-1 $\beta$  cells. Treatment with Z-VAD-FMK inhibited IL-1 $\beta$  secretion in a dose-dependent manner (Figure 1).



**Figure 1: Z-VAD-FMK inhibits the NLRP3 inflammasome response in a dose-dependent manner.**

THP1-Null2 cells, primed with LPS-EK (1 µg/ml for 3 h), were stimulated with MSU crystals (150 µg/ml) and increasing concentrations of Z-VAD-FMK. After overnight incubation, IL-1 $\beta$  secretion was analyzed by adding 50 µl of supernatant from treated THP1-Null2 cells to HEK-Blue™ IL-1 $\beta$  cells. IL-1 $\beta$ -induced activation of NF- $\kappa$ B was assessed by measuring the levels of SEAP in the supernatant of HEK-Blue™ IL-1 $\beta$  cells using QUANTI-Blue™ Solution, a SEAP detection reagent, and by reading the optical density (OD) at 655 nm. Data are shown as percentage (%) inhibition.

## TECHNICAL SUPPORT

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