

# Validation data for Ferrostatin-1

[www.invivogen.com/ferrostatin-1-ferroptosis-inhibitor](http://www.invivogen.com/ferrostatin-1-ferroptosis-inhibitor)

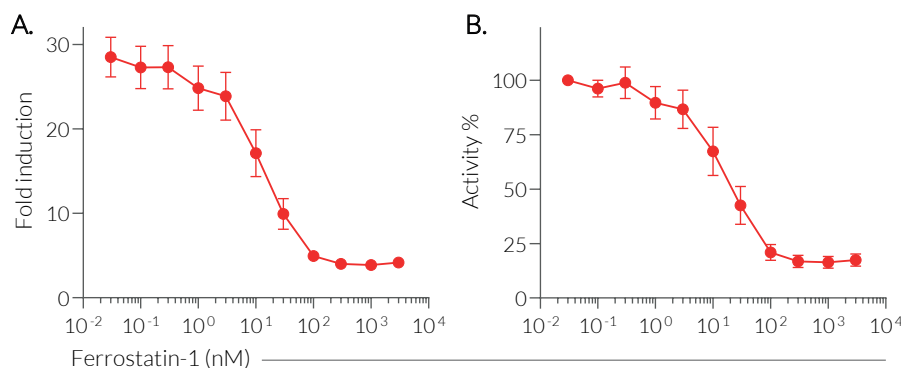
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Version 25C13-AK

Ferrostatin-1 is a strong, synthetic inhibitor of ferroptosis, a form of non-apoptotic cell death triggered by lipid peroxidation. The ability of Ferrostatin-1 to inhibit cell death is verified using InvivoGen's ferroptosis reporter cell line HT-1080-HMGB1-Lucia™.

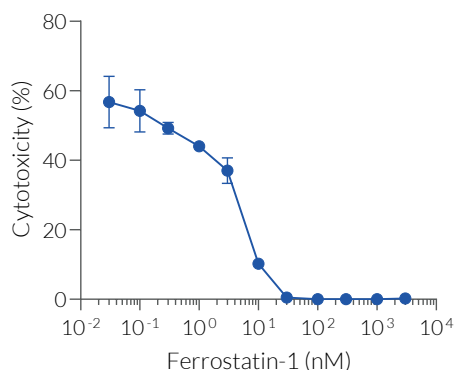
Upon stimulation with RSL3, a potent ferroptosis inducer, the cell membrane ruptures and the reporter protein HMGB1::Lucia is released in the extracellular milieu. Levels of HMGB1::Lucia in the supernatant can be readily monitored using QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia® luciferase detection reagent. Ferrostatin-1 successfully inhibits RSL3-induced ferroptosis and subsequent HMGB1::Lucia release (Figure 1). Moreover, ferroptosis in this cell line can be assessed using the classic cytotoxic lactate dehydrogenase (LDH) assay (Figure 2).

## Inhibition of ferroptosis by Ferrostatin-1 (HMGB1::Lucia readout)



**Figure 1. Ferrostatin-1 inhibits RSL3-induced HMGB1::Lucia release in a dose-dependent manner.** HT1080-HMGB1-Lucia™ cells were incubated with increasing concentrations of Ferrostatin-1 (0.03 nM - 3  $\mu$ M) for 30 min followed by the addition of RSL3 (1  $\mu$ M). After 48 hours, the inhibition of cell death was quantified by measuring the levels of HMGB1::Lucia in the supernatant using QUANTI-Luc™ detection reagent. Data are shown as (A) fold induction over non-induced cells or (B) percentage (%) activity with 100 % equals RSL3 without inhibitor (mean  $\pm$  SEM).

## Inhibition of ferroptosis by Ferrostatin-1 (LDH readout)



**Figure 2. Ferrostatin-1 inhibits RSL3-induced LDH release in a dose-dependent manner.** HT1080-HMGB1-Lucia™ cells were incubated with increasing concentrations of Ferrostatin-1 (0.03 nM - 3  $\mu$ M) for 30 min followed by the addition of the ferroptosis inducer RSL3 (1  $\mu$ M final concentration). After 48 hours, the inhibition of cell death was quantified using the lactate dehydrogenase (LDH) assay. Data is shown as a percentage of cell death (mean  $\pm$  SEM).

### TECHNICAL SUPPORT

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