

# Validation data for Ferrostatin-1

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

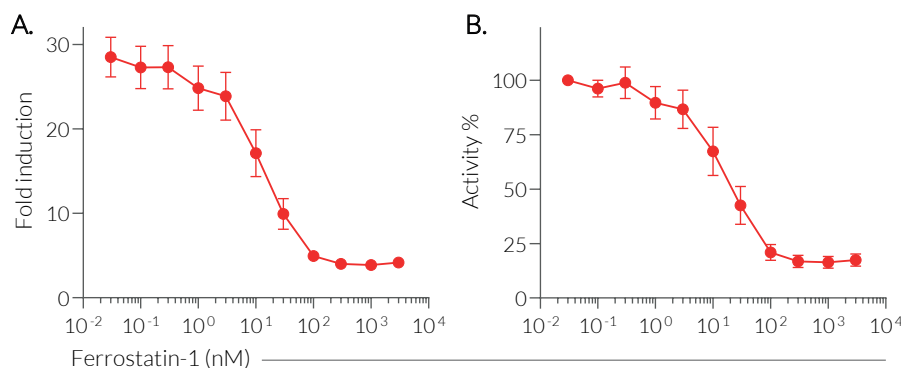
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Version 25C13-AK

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™.

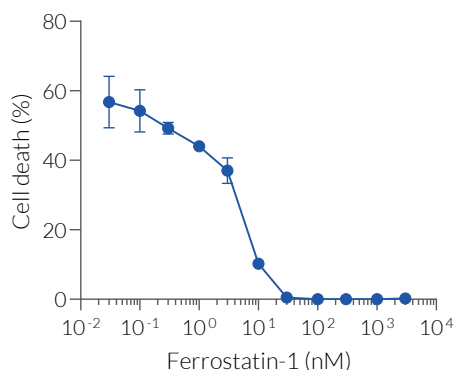
Upon stimulation with RSL3, a potent ferroptosis inducer, the cell membrane ruptures and the reporter protein HMGB1::Lucia is released in the extracellular milieu. Levels of HMGB1::Lucia in the supernatant can be readily monitored using QUANTI-Luc™ 4 Lucia/Gaussia, a Lucia® luciferase detection reagent. 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 μM) for 30 min followed by the addition of RSL3 (1 μM). 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 ± 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 μM) for 30 min followed by the addition of the ferroptosis inducer RSL3 (1 μM 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 ± SEM).

### TECHNICAL SUPPORT

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