Validation data for THP1-HMGB1-Lucia™ cells

https://www.invivogen.com/thp1-hmgb1-lucia

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Version 21G22-MM

THP1-HMGB1-Lucia™ cells derive from the THP-1 human monocytic cell line and are designed to study inflammasome-mediated pyroptosis and necroptosis. These two forms of necrotic programmed cell death are characterized by the release of the alarmin HMGB1 (High Mobility Group Box-1) upon cell membrane rupture. THP1-HMGB1-Lucia™ cells stably express in the cytoplasm a 46.5 kDa fusion protein, in which the C-terminus of HMGB1 is fused to the Lucia luciferase. These cells represent an alternative to the classical lactate deshydrogenase (LDH) release assay to monitor regulated necrosis.

For pyroptosis assays, THP1-HMGB1-Lucia™ cells are typically primed with LPS before treatment with inflammasome inducers, such as Nigericin (NLRP3 inducer) or poly(dA:dT) (AIM2 inducer). Levels of the HMGB1::Lucia fusion protein in the supernatant can be readily monitored by measuring the light signal produced after addition of QUANTI-Luc™. This assay is very sensitive: results display a lower background and a better range of detection than the LDH assay (Figure 1).

For necroptosis assays, THP1-HMGB1-Lucia™ cells are typically treated with a caspase-8 inhibitor, such as Z-VAD-FMK, prior to incubation with a cIAP inhibitor, such as BV6, and TNF-α. As for pyroptosis assays, levels of the HMGB1::Lucia fusion protein released in the supernatant can be readily monitored by measuring the light signal produced after addition of QUANTI-Luc™ (Figure 2).

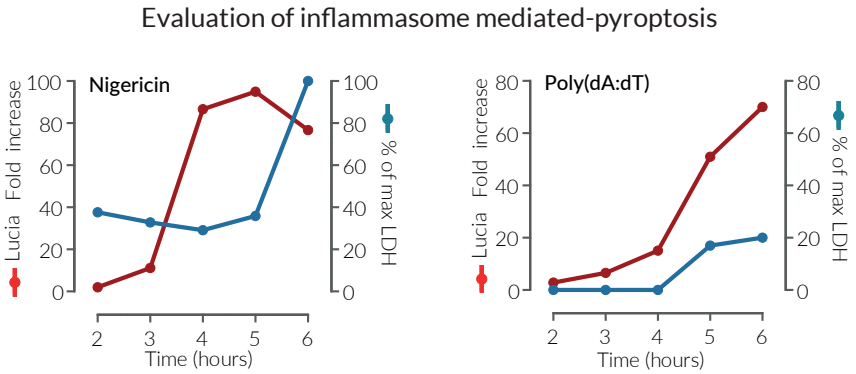

Figure 1: Pyroptosis response of THP1-HMGB1-Lucia™ cells. Cells were primed with 1 µg/ml LPS-EK for 3 hours and then incubated with inflammasome inducers : 8 µM Nigericin (left panel) or 0.5 µg/ml complexed Poly(dA:dT) (right panel). Lucia luciferase activity and LDH release in the supernatant were quantified at 2, 3, 4, 5 and 6 hours post-induction. The Lucia luciferase activity was determined by measuring relative light units (RLUs) in a luminometer using QUANTI-Luc™ detection reagent. Results are presented as the fold increase over non-induced cells. The LDH release was determined using a commercially available LDH cytotoxicity assay. Results are presented as percentage of the maximal LDH release measured in positive control.

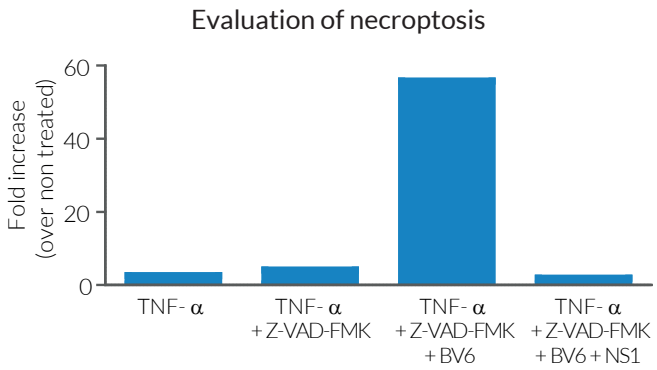

Figure 2: Necroptosis response of THP1-HMGB1-Lucia™ cells. Cells were pre-treated with the pan-caspase inhibitor Z-VAD-FMK (25 µM) for 1 hour prior to incubation with TNF-α (100 ng/ml) alone or in combination with the cIAP inhibitor BV6 (5 µM). As a control of necroptosis induction, Necrostatin 1 (NS1; 30 µM), a necroptosis inhibitor was added to the TNF-α and cIAP mix. Eight hours later, the Lucia luciferase activity was determined by measuring RLUs in a luminometer using QUANTI-Luc™ detection reagent. Results are presented as the fold increase relative to non-treated cells.

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