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We use this protocol and it's working

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Live-cell imaging; cell death assay

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ABSTRACT

This protocol contains instructions for examining cell death in live-cell imaging.

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1 Cell death is detected using Propidium iodide (PI, Thermo Fisher Scientific) or SYTOX™ Green (SYTOX, Thermo Fisher Scientific) which is excluded from viable cells but exhibits red (PI) and green (SYTOX) fluorescence following a loss of membrane integrity and Hoechst 33342 (Hoechst, Thermo Fisher Scientific).

2 20 µM PI or 500 nM SYTOX and 10 uM Hoechst are directly added to the dishes.

3 Cells are incubated for 15 min.

4 Live-cell imaging is performed using a confocal microscope.

Note

Live-cell imaging is performed using a confocal microscope (Zeiss LSM 710 or 880 with an integrated META detection system). For confocal microscopes, illumination intensity is limited to 0.1-0.2% of laser output to prevent phototoxicity, and the pinhole is set to allow optical slice at approximately 1-2 µm.

5 Hoechst and PI are excited by 405 nm and 543 nm laser lines with the emission between 405 nm to 470 nm and 570 nm to 640nm respectively.

6 SYTOX is excited by a 488 nm laser with emissions between 488 nm and 516 nm.