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Live-cell imaging: cell death assay

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Protocol status: Working

We use this protocol and it's working

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Abstract

Examining cell death in midbrain dopaminergic neurons using SYTOX Green



- 1 Cell death is detected using SYTOX™ Green (SYTOX, Thermo Fisher Scientific) which is excluded from viable cells but exhibits green (SYTOX) fluorescence following a loss of membrane integrity.

Hoechst 33342 (Hoechst, Thermo Fisher Scientific) is used as a nuclear cell stain.

- 2 Cells were incubated with 500 nM SYTOX and 10 uM Hoechst for  00:40:00 at room temperature made up in HBSS.

40m

- 3 Live-cell imaging is then performed using a confocal microscope.

- 3.1 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 1% of laser output to prevent phototoxicity, and the pinhole is set to allow optical slice at approximately 1-2 μm .

- 3.2 Hoechst is excited by 405 nm laser line with the emission between 405 nm to 470 nm.

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