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Live-cell imaging: cell death assay

gurvir.virdi[1]

[1]UCL Institute of Neurology

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gurvir.virdi

ABSTRACT

Examining cell death in midbrain dopaminergic neurons using SYTOX Green

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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 40m

temperature made up in HBSS.

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.

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