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Fura-2 imaging of ionomycin response, with and without R568; a CaSR positive modulator

In 1 collection

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ABSTRACT

Fura-2 imaging protocol to accompany Kilfeather, Khoo et al., 2023: **Single cell spatial transcriptomic and translatomic profiling of dopaminergic neurons in health, ageing and disease**

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Protocol status: Working
We use this protocol and it's working

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Data Capture

1 Prepare Fura-2 AM to 5 mM concentration in calcium-free HBSS supplemented with 20 mM HEPES.

2 Dilute Fura-2 AM to the final concentration of 2.5 mM in Neurobasal medium supplemented with B27 and L-glutamine, with DMSO (0.1%) or the CaSR positive modulator R568 (10 mM).

3 Incubate iPSC-derived dopaminergic neurons (DAns) in the solution of Fura-2 AM and drug for 1 h at 37°C, 5% CO_2 and then image on a FlexStation 3 Multi-Mode Microplate Reader (Molecular Devices) at 37°C.

4 Excite the dye at 340 nm and 380 nm and detect at 510 nm. Image each well every 4 s for 100 s and inject with ionomycin (final concentration 5 mM) after a baseline of 28 s.

Analysis

5 For the analysis, compute the 340/380 ratio, and then subtract the baseline from all the timepoints, to obtain a normalised trace for each well. Find the maximum intensity (peak amplitude) of the normalised trace and calculate the area under the curve (AUC) using the left rectangular approximation method.