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Calcium Imaging Protocol for hIPSC-derived Dopaminergic neurons

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SOX6 mDA differentiation

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

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Abstract

This is the imaging protocol to analyse calcium dynamics *in vitro* KOLF2.1J differentiated midbrain dopaminergic neurons

Protocol materials

Fluo-4 Calcium Imaging Kit **Thermo Fisher Catalog #**F10489

Pluronic® F-127 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P2443

Preparation of Loading Solution

1 Prepare the dye-loading solution in Krebs-Ringer buffer:

- 5 µM Fluo-4 AM Fluo-4 Calcium Imaging Kit **Thermo Fisher Catalog #**F10489
- 0.625% Pluronic F-127
  Pluronic® F-127 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P2443

Mix thoroughly to ensure dye solubilization.

Dye Loading

30m

2 Add loading solution to the 35 mm culture dishes containing live cells.

3 Incubate at 37°C for 30 minutes **in the dark**. 37 °C

30m

Washing

4 After incubation, **wash cells twice** with fresh Krebs-Ringer buffer to remove excess dye.

5 Maintain cells in **fresh Krebs-Ringer buffer** at **37°C** during imaging. 37 °C

Calcium Imaging

6 Perform imaging using a Nikon CrEST X-Light V3 **spinning disk confocal microscope**:

- Objective: 20x Air lens
- Excitation wavelength: 480 nm
- Sampling frequency: 1 Hz (one frame per second)

Use **Nikon NIS-Elements software** to control the microscope and acquire time-lapse fluorescence data.

Data Analysis

7 Export image sequences from NIS-Elements.

8 Analyze calcium signals using the following tools:

- FIJI (ImageJ) for image preprocessing and intensity extraction.

Software	
ImageJ/Fiji	NAME
Windows 7	OS
National Institutes of Health	DEVELOPER
http://wsr.imagej.net/distros/win/ij152-win-java8.zip	REPOSITORY
https://imagej.nih.gov/ij/	SOURCE LINK

- MATLAB (R2020b) for custom analysis scripts.

Software	
MATLAB	NAME
Microsoft Windows 10	OS
Mathworks	DEVELOPER
Company Website	REPOSITORY
https://www.mathworks.com/products/matlab.html	SOURCE LINK

- FluoroSNNAP (GitHub: https://github.com/tapan-patel/FluoroSNNAP) for spike detection and activity analysis.