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Calcium imaging in astrocytes

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ABSTRACT

Calcium imaging in astrocytes

PROTOCOL CITATION

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1 Coat a 10 cm plate with 0.1% gelatin for **00:20:00** 20m

2 From a 80% confluent 10 cm plate of astrocytes obtained from protocol https://www.protocols.io/view/astrocyte-extraction-from-brain-organoids-261ge364wl47/v2

3 Add 3 mL of
TrypLE™ Select Enzyme (1X), no phenol red **Thermo Fisher Catalog #12563029**
for
00:05:00
5m

4 **300 rcf, 25°C, 00:03:00**
3m

5 Add pTALV-fUBIGW-GCAMP8SIRES-PURO lentivirus 1/100 in
Astrocyte
Medium **ScienCell Catalog # #1801**
to the pellet and resuspend the cells

6 Incubate **00:05:00**
5m

7 Add to a gelatin coated 10 cm plate with a 9 ml
Astrocyte
Medium **ScienCell Catalog # #1801**
warmed media.

8 **48:00:00** observe fluorescence levels to confirm infection
2d

9 Wait **168:00:00** for the GCAMPs expression to reach stable levels
1w

10 Coat a 2 cm plate with 0.1% gelatin for **00:20:00**
20m

11 Plate 50k astrocytes of infected astrocytes

12 after **48:00:00** change to mature astrocyte media
2d

13 Before the start of imaging aim for 80% confluency.

14 Place the plate on a

Nikon Eclipse TE2000-U microscope
Microscope
Nikon **N/A**

plate holder.

15 Continuously perfused with ACSF with the following composition (in mM): NaCl 125, KCl 5, D-Glucose 10, HEPES-Na 10, $CaCl_2$ 3.1, $MgCl_2$ 1.3. using a ValveBank8 II controller.

16 Using a 10X objective. GCaMP8s was excited using a 480 nm (Mic-LED-480A, Prizmatix), an HQ480/40x excitation filter, a Q505LP dichroic mirror, and an HQ535/50m emission filter (Semrock).

17 Sample at a rate of 4.7 fps with a frame exposure of 200 ms at 160x120 pixels (4x4 binning).

18 ROI segmentation of GCaMP8s, raw fluorescence extraction, and background correction can be performed with Nikon Elements software.