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Calcium imaging in astrocytes

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Protocol status: Working

We use this protocol and it's working

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Abstract

Calcium imaging in astrocytes

Protocol materials

✕ TrypLE[®]; Select Enzyme (1X), no phenol red **Thermo Fisher Catalog #12563029**

✕ Astrocyte Medium **ScienCell Catalog # #1801**



- 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

Equipment

Nikon Eclipse TE2000-U microscope	NAME
Microscope	TYPE
Nikon	BRAND
N/A	SKU

plate holder.

15 Continuously perfused with ACSF with the following composition (in mM): NaCl 125, KCl 5, D-Glucose 10, HEPES-Na 10, CaCl₂ 3.1, MgCl₂ 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 160×120 pixels (4×4 binning).

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