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Live Cell Imaging

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We use this protocol and it's working

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Abstract

Protocol for preparing cells for live-cell imaging

Preparing Astrocytes

1 Refer to the following protocols for midbrain astrocyte differentiation:
10.1016/j.xpro.2021.100463
https://www.protocols.io/view/astrocyte-extraction-from-brain-organoids-261ge364wl47/v2

2 Refer to the following protocols for astrocyte lentiviral transduction:
https://protocols.io/view/astrocyte-viral-transduction-gzhtbx36p

Plating Astrocytes

3 No fewer than 3 days after lentiviral transduction, and after completion of antibiotic selection, passage astrocytes into plate for imaging

3.1 Coat desired wells of a 96-well, black plate with cover-slip thickness plastic (Greiner **655096** or similar) with 0.1% gelatin

3.2 Wash astrocytes with 1x PBS

3.3 Dissociate astrocytes in a trypsin enzyme solution (TrypLE Select, Gibco 12563011). For a 6-well plate of astrocytes, use 1mL per well

3.4 Transfer dissociated cells to a falcon tube with 5mL astrocyte media (AM; ScienCell #1801)

3.5 Centrifuge at 300rcf for 3 minutes at room temperature

3.6 Resuspend the cell pellet in 1mL AM and count cells

3.7 Plate 1×10^4 cells per well of the precoated 96-well plate

Imaging Astrocytes

4 Approximately 3 days later, incubate the cells with a live-cell compatible nuclear dye (such as Hoechst33342 or DRAQ5) for 10 minutes

5 Wash the cells 1x in PBS and replace with fresh AM media

6 Acquire images on a microscope equipped with an incubation unit and maintain the temperature at 37°C, CO_2 at 5%, and humidity at 60%