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Immunofluorescence and live-cell Imaging

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We use this protocol and it's working

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Abstract

This protocol contains a detail description of how to perform immunostaining on two different cell types, U2OS and iPSCs cells.
It also describes how to perform live-cell imaging procedure using a Zeiss LSM900 confocal microscope in a temperature-controlled environment.

Troubleshooting

Immunofluorescence of U2 cells

50m

1 U2OS cells were washed once with PBS and immediately fixed by 4% EM-grade paraformaldehyde for 00:10:00 at Room temperature — 10m

2 Cells were washed three times with PBS for 00:10:00 each time. — 10m

3 Blocked and permeabilized for 00:30:00 in permeabilization buffer (5% FBS and 0.1% saponin in PBS) — 30m

4 Cells were then incubated with 1:100 dilution of primary antibodies at 4 °C Overnight — 1h

5 Cells were washed three times with PBS for 00:10:00 each time. — 10m

6 Cells were incubated with 1:500 dilution of fluorophore-conjugated secondary for 00:30:00 at Room temperature — 30m

7 Prolong Gold with DAPI was used as mounting solution

8 Images were acquired with a Zeiss LSM900 confocal microscope and analyzed with Fiji/ImageJ software

Immunofluorescence of hiPSC dopamine neurons

2h 40m

9 Cells were fixed with 4% paraformaldehyde in PBS and 0.1% Triton-X was used for permablization 00:10:00 — 10m

10 Blocked in 10% normal donkey serum for 01:00:00 Room temperature — 1h

11 Cells were then incubated with 1:100 dilution of primary antibodies at 4 °C Overnight — 1h

12 Cells were washed three times with PBS for 00:10:00 each time. 10m

13 Cells were incubated with 1:500 dilution of fluorophore-conjugated secondary for 00:30:00 at Room temperature 30m

14 Prolong Gold with DAPI was used as mounting solution

15 Images were acquired with a Zeiss LSM900 confocal microscope and analyzed with Fiji/ImageJ software

Live-cell imaging 15m

16 Cells were cultured in 35 mm glass bottom dishes (MatTek).

17 HaloTag fluorescent ligands were added according to the manufacturer's protocol (Promega).

18 After incubation for 00:15:00 in the incubator (37 °C and 5% CO2), the cells were quickly washed twice with PBS. The medium was replaced with Opti-MEM supplemented with 10% FBS. 15m

19 Imaging was performed using a Zeiss LSM900 confocal microscope in a temperature-controlled
(37 °C and 5% CO2) environment.