



AUG 26, 2023

OPEN ACCESS



DOI:
dx.doi.org/10.17504/protocols.io.bp2l6x9b1lqe/v1

Protocol Citation: Shenjie Wu, Nancy C. Hernandez Villegas, Iona Thomas-Wright, Richard Wade-Martins, schekman 2023. Immunofluorescence and live-cell Imaging. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.bp2l6x9b1lqe/v1>

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

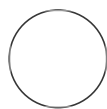
🌐 Immunofluorescence and live-cell Imaging

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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.


Created: Aug 03, 2023

Last Modified: Aug 26, 2023

PROTOCOL integer ID:
85900

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 30m
at  Room temperature
- 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.