Immunofluorescence

Introduction

Standard protocol for immunofluorescence.
Updated: 07/02/2020.

Materials

- Sample on coverslips
- 100% MetOH (at -20°C)
- PBS
- PBST
  - PBS supplemented with 0.05%(v/v) Tween20
- Blocking buffer
  - PBST-BSA: PBST supplemented with 3%(w/v) BSA (Make fresh)
- Antibodies diluted in PBST-BSA
- Hoechst (use 1:2000 dilution)
- Mounting medium
  - Fluoromount G
  - Glycerol based mounting medium (homemade stored at -20°C)
  - Vectashield
  - TDE
- Glass slides

Procedure

Fixation

1. Aspirate the medium from the cells and add -20°C cold MetOH. For 6-well plate add ~2mL.

   Note: For pre-extraction of cytosolic proteins, add 1xPBS+0.5%(v/v) Triton X1000 to each well for ~30sec. Then proceed to add MetOH.

2. Incubate at -20°C for 7min.

3. Remove MetOH and wash 3X with PBS.

4. Store plate at 4°C until further processing (cells should be OK for a few weeks).

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5. Assemble a humidity chamber covered with aluminium foil.
6. Label a plate-cover with condition then wrap it with Parafilm and place it in the humidity chamber.
7. Place coverslips on the Parafilm and add PBST on top. Avoid leaving cells without any liquid.

    Note: if cells have been fixed with PFA, incubate for 30min with PBST to permeabilise.

8. Remove PBST and add blocking buffer (~200µL per coverslip).
9. Incubate at RT for 30min.

00:30:00

10. Prepare primary antibody dilutions in blocking buffer.

    Note: For antibody dilutions see antibody list.

11. Aspirate blocking buffer and add primary antibody mixture. Add ~200µL for 15mm and 150µL for 12mm coverslips.
12. Incubate at RT for a few hours or overnight in the cold room.
13. Wash 4X with PBST. The first wash right away and the other 3 with 5min incubations. *[Alternatively, use the box with porcelain holder; place coverslips in for 5 min and proceed to next step}*

00:05:00

00:05:00

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14. Prepare secondary antibody dilutions in blocking buffer. Typical dilution is 1:1000.
15. Aspirate PBST and add secondary antibody mixture. Add ~200µL for 15mm and 150µL for 12mm coverslips.
16. Incubate for 45min at RT.

00:45:00

17. Wash with PBST one time right away.

18. Wash with PBST with 5min incubation.

00:05:00

19. Wash with PBST + Hoechst (1:2000) for 5min.

00:05:00

20. Wash with PBST right away and then with 5min incubation.

00:05:00

21. Prepare glass slides with labels for the coverslips.

22. Dip coverslip in MQ and blot coverslip with paper to remove as much liquid as possible.

23. Place on a glass slide with 3µL mounting medium.

24. Remove excess liquid at the sides of the coverslip with a tissue.

25. Add nail polish to one side to stabilise the coverslip. When dry, seal with nail polish all around.

    Note: this step if not needed if Fluoromount-G is used.

26. Place in a slidebook, add clear label on the outside and store in the cold room.