**Immunofluorescence**

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**Abstract**

This protocol describes the fixation of cultured cells in paraformaldehyde solution and immunofluorescent labeling of mitochondria and DNA.

**Keywords**

Immunofluorescence, HSP60, DNA, spinning-disk confocal microscopy

**Solutions to prepare**

**DMEM** containing 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (all from Gibco).

**Fixative solution:** 4% paraformaldehyde in PBS.

**Permeabilization solution:** 0.1% (v/v) Triton X-100 in PBS

**Blocking solution:** 1% (w/v) BSA in PBS, filtered through 0.45 micron filter.

**Primary antibody solution**: PBS containing 1% BSA and antibodies against DNA (CBL186, EMD Millipore, 1:150) and HSP60 (12165S, CST, 1:1000)

**Secondary antibody solution:** Add filtered PBS containing 1% BSA and primary antibodies against DNA (CBL186, EMD Millipore, 1:150) and HSP60 (12165S, CST, 1:1000).

**Protocol**

1. Plate 35,000 WT or VPS13CKO HeLa cells on 22 x 22 mm glass coverslips in 6-well dishes in DMEM containing 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 2 mM L-glutamine (all from Gibco).
2. Incubate overnight at 37°C in 5% CO2
3. Pre-warm a solution of 4% paraformaldehyde in PBS to 37°C.
4. Aspirate media from cells and immediately proceed to step 5.
5. Add pre-warmed 4% paraformaldehyde to cells.
6. incubate for 15 minutes at room temperature.
7. Remove 4% paraformaldehyde solution and dispose of it in an appropriate chemical waste container.
8. Rinse each well with 1 mL PBS. Remove rinse and dispose of it in an appropriate chemical waste container.
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10. Rinse each well with 1 mL PBS. Remove rinse and dispose of it in an appropriate chemical waste container.
11. Add 0.1% (v/v) Triton X-100 in PBS and incubate for 10 minutes at room temperature to permeabilize cells.
12. Aspirate Triton X-100 solution.
13. Add filtered PBS containing 1% (w/v) BSA and incubate for an hour at room temperature to block nonspecific antigens.
14. Aspirate BSA solution.
15. Add filtered PBS containing 1% BSA and primary antibodies against DNA (CBL186, EMD Millipore, 1:150) and HSP60 (12165S, CST, 1:1000).
16. Incubate at 4°C overnight.
17. Aspirate antibody solution and wash cells with PBS for 5 minutes.
18. Repeat wash with PBS for 5 minutes.
19. Repeat wash with PBS for 5 minutes.
20. Add filtered PBS containing 1% BSA and secondary antibodies (1:1000, Alexa fluorophores 488 and 555, Invitrogen).
21. Incubate at room temperature for 1 hour.
22. Aspirate antibody solution and wash cells with PBS for 5 minutes.
23. Repeat wash with PBS for 5 minutes.
24. Repeat wash with PBS for 5 minutes.
25. Rinse coverslips in milliQ water.
26. Mount coverslips onto slides using ProLong Gold Antifade Mountant with DAPI (ThermoFisher P36935).
27. Allow slides to cure overnight in darkness.
28. Image.