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Confocal Microscopy of DFP-Treated Cells

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

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Abstract

This protocol details the confocal microscopy of DFP-Treated cells after fixation and staining.

Materials

- Bovine Serum Albumin [BSA] **Fisher Scientific Catalog #**9048-46-8
- DAPI Fluoromount-G® **Southern Biotech Catalog #**0100-20

Procedure

1d 2h 20m

1 Seed the cells on glass coverslips (12 mm #1.5) at a concentration of 120,000 cells/well, and after treatment with DFP for 24:00:00, fix in 100% methanol for 00:20:00 at -20 °C. 1d 0h 20m

2 After washing with PBS, block the samples with 5% (v/v) BSA (9048-46-8, Sigma-Aldrich) diluted in PBS for 01:00:00 at Room temperature. 1h

3 Dilute the primary and secondary antibodies in 5% BSA and incubate for 01:00:00 at Room temperature with three PBS washing steps in between. 1h

4 Mount the cells on microscopy slides in DAPI Fluoromount-G mounting medium (0100-20, Southern Biotech), which stains the nuclei, add a cover glass, and store at 4 °C until use.

5 Perform the confocal microscopy with a Zeiss LSM700 laser scanning confocal microscopy with Plan-Apochromat 40×/1.30 Oil DIC, WD 0.21 mm objective or Zeiss LSM900 with Airyscan 2 confocal super-resolution microscopy with Plan-Apochromat 63×/1.4 Oil DIC, WD 0.19 mm objective.