



Cytoskeleton-Tubulin Fixation

This protocol can be used to prepare cell culture samples for super-resolution microscopy applications.

Cytoskeletal Buffer (CBS)

- 10 mM MES pH 6.0 ACE MES buffer
Amresco K458-100 mL
- 138 mM KCL Sigma P-9333
- 3 mM MgCl Sigma M2670-500
- 2 mM EGTA Sigma E8145-10G
- 0.32M sucrose

Add sucrose fresh on the day of use of the buffer from 4°C stock. Sucrose formula weight is 342.3 g/mol.

Materials

- PBS (pH ~7.4)
- Cytoskeletal buffer (CBS)
- Paraformaldehyde (PFA-16%): Electron Microscopy Services #15710
- Glutaraldehyde (Glut-8%): Electron Microscopy Services #16019
- Saponin-USB Corporation #21435 100 gram
- Triton X-100 (TX-100)
- Bovine Serum Albumin (BSA): Sigma A3059-10G
- Primary antibody: α -Tubulin anti-mouse monoclonal antibody (Sigma T5168-.2ML)
- Secondary antibody: Alexa Fluor® 647 goat anti-mouse IgG (H+L) (Invitrogen-A21236)
- Adherent cells grown on No: 1.5 coverslips: BSC-1, Cos-7, or Ptk2 cells work well

Related Resources

<https://www.bruker.com/en/products-and-solutions/fluorescence-microscopy/super-resolution-microscopes/cell-biology-microscopes.html>

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Reagents

- Fixation and post-fixation buffer: 3% PFA+0.1% glutin CBS
- Pre-extraction buffer: 0.2% saponin (w/v) in CBS
- Blocking buffer: 3% BSA+0.2% TX-100 in PBS
- Antibody dilution buffer: 1% BSA+0.2% TX-100 in PBS (used for diluting both primary and secondary antibodies)
- Wash buffer: 0.05% TX-100 in PBS

Procedure

1. Wash cells three times with PBS warmed to 37°C.
2. Pre-extract with 0.2% saponin in CBS (warmed to 37°C) for 1 minute at room temperature.
3. Do not rinse, just aspirate the saponin mixture.
4. Fix with Cytoskeletal buffer 3% PFA +0.1% glutaraldehyde diluted in CBS (warmed to 37°C) for 15 minutes at room temperature.
5. Rinse 3x3 minutes with PBS.
6. Permeabilize and block with blocking buffer for 30 minutes at room temperature gently rocking.
7. Aspirate but do not rinse.
8. Incubate with primary antibody (for microtubules ~1 μ g/mL concentration) for 1 hour at room temperature gently rocking.
9. Rinse 3x3 minutes at room temperature with wash buffer.
10. Incubate with secondary antibody (e.g., with Alexa dyes, we use ~5 μ g/mL concentration) for 1 hour at room temperature gently rocking wrapped with aluminum foil.
11. Rinse 3x3 minutes with wash buffer.
12. Post fix for 10 minutes at room temperature.
13. Rinse 3x3 minutes with PBS.
14. Store cells in PBS at 4°C.