

QUICK DAPI STAINING OF YEAST

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****FORMALDEHYDE FIX CELLS PRIOR TO CENTRIFUGATION** to avoid displacement of nucleus. Centrifugation of live cells will also disrupt the actin cytoskeleton.

1. Add 1/10 volume 37% formaldehyde to an aliquot of cell culture. Fix at 23°C for ≤ 2h.
2. Spin down cells (1-2 min at 2K); wash 2x with PBS (or H₂O).
3. Resuspend cells in 300 μ L PBS (or H₂O). Cells can be stored at 4°C at this point. Stop here if you intend to wait a long time before scoring cells.
4. Add 700 μ L 100% Ethanol → final concentration is 70% EtOH.
5. Let sit at room temperature (or on ice) for 30-40 minutes.
6. Spin down cells.
7. Resuspend in PBS (or H₂O). Can be stored at 4°C at this point.
8. Lightly sonicate the cells prior to observation (7 pulses at 1.5 output/20% duty cycle).
9. Place a small volume of EtOH-permeabilized cells (e.g. 8 μ l) on microscope slide. Mix with an equal volume of mounting medium containing DAPI (50-100 ng/ml). Be sure to pipet up and down to mix well. Put on a coverslip and observe. No need to seal the edges.