

Preparation of yeast for electron microscopy

1. Grow cells in YPD, inoculate fairly early in the day prior to when you want them ready. Never let them grow over 0.5 O.D./ml. Harvest ~100ml (i.e. about 50 O.D. total) by spinning in the clinical centrifuge setting 3 (210 x g) for 10 min.
2. Resuspend in 2 ml fix buffer (i.e. 3% glutaraldehyde, 0.1 M NaCacod pH 7.4, 5 mM CaCl₂, 5 mM MgCl₂, 2.5% sucrose) and fix for 1 hour at 25 degrees with gentle agitation.
3. Spin cells in eppendorf setting 6 for 2 min.
4. Wash in 100mM cacodylate, pH 7.4.
5. Wash once with 1 ml TDES.
6. Resuspend in 1 ml TDES and incubate at room temperature for 10 min.
(Softens cell wall)
7. Spin cells setting 4 for 2 min.
8. Wash 1 ml 0.1 M phos-citrate/1 M sorbitol.
9. Resuspend in 0.5 ml phos-citrate/sorbitol. Add 50ul B-glucuronidase, 25 ul of 10 mg/ml zymolyase. Incubate at 30 degrees for 30-45 minutes with occasional agitation. Increase the amount of zymolyase and/or incubation time for mutants with thick cell walls.
10. Spin cells setting 4 for 2 min.
11. Wash in 1 ml 0.1 M cacodylate/ 5mM CaCl₂/ 1M sorbitol.
12. Resuspend in 0.5 ml cacodylate/CaCl₂/ 1M sorbitol, place on ice and follow with osmium-thiocarbohydrazide-osmium staining.

TDES (10 ml)

1 ml Tris (1M, pH 7.5)
0.25 ml DTT (1M)
0.1 ml EDTA (0.5M)
6 ml sorbitol (2M)
2.65 ml H₂O

0.2M phos-citrate (100 ml, 2X stock)

K₂HPO₄ 3.48g (or 4.56g K₂HPO₄•3H₂O)
citric acid 1.4g

Should be ~pH 5.9 Mix this 1:1 with 2M sorbitol for the working solution

0.5M NaCacodylate (pH 6.8) / 25mM CaCl₂ (80 ml, 5X stock)

1M NaCacodylate pH 6.8 40 ml
1M CaCl₂ 2 ml
H₂O 38 ml

Osmium-Thiocarbohydrazide-Osmium Staining

1. Disperse cells and embed in 2% ultra low temperature agarose (made in water). Cool. Cut into small pieces (~1mm³).
2. Post-fix blocks in 1% OsO₄/ 1% potassium ferrocyanide in 0.1M cacodylate/ 5mM CaCl₂, pH 6.8. Fix at room temperature for 30 minutes.
3. Wash blocks thoroughly (4X in ddH₂O, 10 minutes total).
4. Transfer blocks to 1% thiocarbohydrazide at room temp. for 5 minutes. (Make 1% thiocarbohydrazide in water, stir for one hour, let undissolved material settle out, and use the solution for the incubation with blocks.)
5. Wash blocks in ddH₂O (4X, 1 minutes each).
6. Transfer blocks to 1% OsO₄/ 1% potassium ferrocyanide in cacodylate buffer pH 6.8 (5 minutes at room temp.).
7. Wash cells 4X with ddH₂O (15 minutes total).
- *8. (OPTIONAL) Use 1-2% tannic acid here for 15-30' for optimal visualization of membrane coats, filaments, etc.
9. Wash cells 4X with ddH₂O (15 minutes total).

En bloc stain in Kellenberger's UA (uranyl acetate) for 2hr to overnight.

11. Dehydrate through a graded series of ethanol. Carryout 50% to 100% on ice (3-5 min/wash); then follow with 4ea 15' washes in 100% at room temp.
12. Transfer blocks to 1:1 ethanol/propylene oxide (10 minutes).
13. Transfer blocks to 100% propylene oxide (2X 5 minutes).
14. Transfer blocks to 1:1 propylene oxide/Spurr or Epon resin (Hard formulation) for overnight under vacuum.
15. Transfer blocks to fresh Spurr or Epon resin, leave for 4-6 hours.
16. Transfer to beam capsules and polymerize in fresh Spurr resin overnight (18-24 hours). Section and post-stain with lead and uranyl acetate.

*Remember: If emphasis on coat proteins/filaments is desired, insert a tannic acid incubation (between steps 7 and 9 after the second osmication, before the uranyl acetate).

After the water washes, incubate in 1-2% tannic acid/100mM cacodylate pH 7.4 for 30 minutes at room temperature. The standard is to use 1% tannic acid, but you can increase it to 2%. If 2% tannic acid is used, check the pH of the solution; you will need to adjust it back to pH 7.4. Finally, again wash thoroughly with water prior to the UA incubation.