PREPARATION OF YEAST CELLS FOR IMMUNO-ELECTRON MICROSCOPY
version 5.0

A) FIXATION WITH ADDED OSMOTIC SUPPORT

1) Grow cells to early log phase (OD_{600} of 0.5 - 0.6) in YEPD or SD medium (2% glucose). Typically we process 100 ml per sample; this will produce at least three blocks of material for sectioning.

2) Quickly harvest cells over a disposable 0.45 um filter unit with a vacuum of -20 to -15 in. Hg. Swirl while filtering to a **final volume of 5 ml**, **do not filter dry**. Disconnect from the vacuum and quickly add 25 ml of fixative directly to the cells. Quickly swirl to cover cells, and use a 10 ml disposable pipet to resuspend the cells by pipetting up and down.

3) Transfer the cell suspension to 50 ml conical tubes and incubate 60 minutes at room temperature.

Fixative: 0.04 M Potassium phosphate buffer (pH6.7)
0.8 M Sorbitol
4% Formaldehyde (make fresh, see below)
0.4% Glutaraldehyde (EM grade, Polysciences, Snap vial)
1 mM $MgCl_2$
1 mM EGTA

Final pH should be between 6.5 and 7. Check it.

These are the final concentrations for the fixative*, i.e. at 30ml which is 5ml of sample plus 25ml of fixative. Therefore, calculate amounts based on 30ml per sample but bring volume of fixative to 25ml, sample will provide 5ml to bring the final volume to 30ml.*

Fixative is made within 1 hr. of processing cultures and divided into 25ml aliquots in 50ml polypropylene conical centrifuge tube (Corning).

Less than 0.4% (e.g. 0.4 to 0.2%) glutaraldehyde may be used for improved antigenic preservation. However, one must be cautious about loss of morphology as well as changes in antibody localization patterns due to extraction of proteins etc. during subsequent steps.

NOTES

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A) FIXATION CONTINUED

Formaldehyde stock (weigh and prepare in a hood)
8 g paraformaldehyde (Polysciences)
15 ml H_2O
Heat to 65 - 70^oC

Add 0.5 - 1 ml 5 N NaOH (freshly made, less than 1 month old)
Shake - solution should clear.

Measure volume and calculate concentration, it should be 37 to 40 %.
Add required amount of formaldehyde stock to fixative.

B) SODIUM METAPERIODATE TREATMENT

4) Pellet cells in a clinical centrifuge (IEC on setting 5 for 2 -3 minutes or in Sorval at 3-4 k rpm, SS34 rotor for 5 minutes) at room temperature (RT) - avoid pelleting the cells too densely. Pour off supernatant.

5) Resuspend and wash cells in the following solutions. Pellet cells at RT as above:
1X (10 ml) - 0.50 M sorbitol in 0.04 M potassium phosphate buffer (pH 6.7)
1X (10 ml) - 0.25 M sorbitol in 0.04 M potassium phosphate buffer (pH6.7)
1X (5 ml) - 0.04 M potassium phosphate buffer (no sorbitol) (pH6.7)
transfer cells to disposable glass test tubes
(13 X 100mm) with a Pasteur pipet prior to pelleting at step 6.

6) Pellet and Resuspend cells in 5 ml of 1% sodium metaperiodate ($NaIO_4$). Make solution just prior to incubation
Incubate 10 - 15 minutes at RT.

Cell pellets can easily be resuspended by gently mixing with a wooden dowel (ASP# A5000-1). Cell clumps should be <1mm.

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C) AMMONIUM CHLORIDE TREATMENT

7) Pellet and wash cells at RT.
1X ddH_2O or Milli-Q H_2O (5 ml).

8) Pellet and resuspend cells in 5 ml of 50 mM ammonium chloride (NH_4Cl).
Incubate 15 minutes RT.

D) DEHYDRATION

9) Pellet and wash cells at RT.
1X in 5 ml ddH_2O or Milli-Q H_2O .

10) Pellet and resuspend cells in ice-cold, ethanol / H_2O series: (5 ml each)
1X - 50% EtOH
1X - 70% EtOH
1X - 80% EtOH
1X - 85% EtOH
1X - 90% EtOH
1X - 95% EtOH
2X - 100% EtOH
1X - 100% EtOH - this step at RT, use a fresh bottle of RT EtOH.

Dehydrations should be done for 5 minutes/step and on ice with ice cold ethanol (except at final 100% step as noted above). Centrifugations can be done at RT.

Do not pellet cells too densely. Pellet should be a little loose; try 1' in IEC clinical on next to the highest setting (6). Again use wooden dowels to resuspend cells in EtOH.

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E) INFILTRATION

11) Pellet cells and pour off EtOH.
Resuspend in **2 ml of a 2:1 mixture of EtOH : LR White resin** (Polysciences)
Place on a roller for 1 hr. at RT.

12) Pellet cells and pour off EtOH / resin.
Resuspend in **2 ml of 1:1 EtOH : LR White resin.**
Place on roller over night at RT.

13) Pellet cells and pour off EtOH / resin.
Resuspend in **1 ml of 1:2 EtOH : LR White resin.**
Place on roller 1- 2 hr.s at RT.

14) Pellet cells and pour off EtOH / resin.
Resuspend in **1 ml 100% LR White resin.**
Place on roller 1 hr. at RT.

15) Pellet cells and aspirate resin with a Pasteur pipet.
Resuspend in **1 ml 100% LR White resin.**

F) EMBEDDING

16) Transfer cells and resin with a Pasteur pipet to gelatin capsules (Ted Pella), capsules should be about 1 / 2 full. A 100ml culture at an OD_{600} of 0.6 should produce enough material for 3 capsules.

17) Top off the capsules with fresh LR White resin, add labels and gelatin caps. Allow cells to settle to bottom of capsules for approximately 15 to 30 minutes, at RT. Place capsules in a heat block set at 47ºC. Cover heat block with lead or several layers of aluminum foil to keep the temperature stable. Polymerize for 2 days at 47ºC.

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G) SECTIONING

Section blocks on a suitable ultramicrotome. Sections should be silver in interference color, corrosponding to a thickness of between 50 to 70 nm. The thinnest sections which still provide good contrast are best. Thin sections provide the best resolution and seem to hold up very well in the microscope.

H) IMMUNOLABELING AND POSTSTAINING

Immunolocalizations should be done as described by **R. Wright and J. Rine, 1989**

After immunostaining, post-fix sections with 8% glutaraldehyde (EM grade, filtered over 0.45 um syringe filter) for 60 minutes. Wash for 60 seconds by gently slicing the grids through 500 ml of mill-Q or double distilled water. We have found that postfixation is necessary to prevent reduction or complete loss of immunolocalization of some antibodies during post staining in high pH (13) lead citrate (see below). This post-fixation also appears to fix the cell sections so that they hold up better under the electron beam.

Sections should be poststained with 2% uranyl acetate (passed through a 0.22-um filter prior to use) for 1 to 2 hours at rt., wash by slicing thru water (milli-Q or distilled) as described above for 15 - 30 seconds. After drying the sections may be stained with Reynolds lead citrate for 15 - 30 seconds, wash as for uranyl acetate. For optimum contrast do not over wash

More info can be found in **R. Wright and J. Rine, Methods in Cell Biology, (1989) 31:473-512**, and, **E.van Tuinen and H. Riezman, J. Histochem. and Cytochem., (1987) 35:327-333.**

Should you have questions:

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