

Yeast Immunofluorescence

Cell Fixation

1. Grow up 10 ml of cells to mid-log phase (make sure you grow an isogenic strain that lacks the antigen of interest if available)
2. Transfer cells to 15 ml conical tube and harvest by centrifugation (3 min. at setting ½, or 3000 rpm)
3. Resuspend in 1 ml 4% paraformaldehyde solution
4. Rock/rotate cells for 10-60 min. at room temperature (the time of fixation is antigen and strain dependent, so generally do a time course of 10', 20', 40', 60' with a new antibody; for Tub4/Cdc31 and microtubules, fix for 15-30')
5. Pellet cells and wash 2x in 5 ml 0.1 M KPO₄ and 1x in 0.1 M KPO₄/1.2 M sorbitol
6. Resuspend cells in 1 ml 0.1 M KPO₄/1.2 M sorbitol
7. Cells can be used immediately or stored at 4 degrees for several days

Spheroplasting

1. Remove 0.5 ml of fixed cells to 1.5 ml eppie
2. Add 10 microliters of beta-mercaptoethanol
3. Add 5 microliters of 10 mg/ml zymolase (spin 5 min. at top speed in microfuge before addition to pellet insolubles)
4. Incubate cells until spheroplasted, generally 10-15 min.
 - A. monitor spheroplasting on microscope—cells should be dark translucent gray when adequately spheroplasted and should lyse when a drop of water is added
 - B. monitor spheroplasting by sensitivity to SDS lysis—put 10 microliters of cells into 0.5 ml 1% SDS at t=0 and measure OD₆₀₀. Remove 10 microliters of cells at 5', 10', 20' etc. and add to 0.5 ml 1% SDS and measure OD₆₀₀.
When the OD₆₀₀ has dropped 60-80%, cells are adequately spheroplasted
- ** note that when doing a fixation time course, cells fixed for different amounts of time will spheroplast at different rates, so monitor both the 10' and 60' time points
5. Spin cells 1 min. 5K in microfuge
6. Wash 2x with 1 ml 0.1 M KPO₄/1.2 M sorbitol, resuspending cells gently with blue tip for each wash (do not vortex)
7. Resuspend cells in 50-200 microliters of 0.1 M KPO₄/1.2 M sorbitol
8. Cells can be stored overnight at 4 degrees at this point

Slide Preparation

1. Chill methanol in coplin jar or other container at -20 degrees well ahead of time
2. Fill another coplin jar with acetone and keep at room temperature
3. Wash chamber slide with 95% EtOH and let air dry
4. Place 15 microliters of 0.1% poly-lysine into each well
5. Let set 5-10 min. at room temperature
6. Wash 3x with ddH₂O then let air dry
7. Place 15 microliters of spheroplasted cells into each well (make sure you have enough wells for controls too)

8. Let set 5-10 min. at room temperature
9. Aspirate supernatant of cells
10. Immediately plunge into ice cold methanol for 5-6 min.
11. Remove slide and immediately submerge in acetone for 30 sec.
12. Quickly air dry slide
13. Immediately add 25 microliters of PBS-BSA to each well of slide
14. Do not let cells dry out until they are mounted

Antibody Staining

1. While the cells are blocking (10-30 min.), prepare primary antibody solutions by diluting antibody in PBS-BSA and spinning 5 min. at full speed in microfuge (if using a new antibody, do a series of 3-5 fold serial dilutions; use only affinity purified polyclonal antibodies as most rabbits have had yeast infections; use 1:500 YOL1/34 for microtubules, 1:1000 anti-Cdc31, 1:500 anti-Tub4, 1:1000 anti-GFP; sometimes adding 0.1% Tween-20 helps expose antigen)
2. Aspirate off block
3. Add 25 microliters of primary antibody to each well
4. Incubate overnight at room temperature in humid chamber (put lightly wet paper towels into a Tupperware container)
5. Prepare the secondary antibody by diluting antibody in PBS-BSA and spinning 5 min. at full speed in microfuge (for FITC & rhodamine/Texas Red antibodies 1:500 dilutions work well; for Cy3 antibodies use at least a 1:2000 dilution)
6. Aspirate off primary antibody
7. Wash wells 5x with PBS-BSA
8. Add 25 microliters of secondary antibody to each well
9. Incubate 1-2 hours in humid chamber in the dark at room temperature (put in drawer or cover with foil)
10. Prepare the DAPI by diluting 1 microliter 1 mg/ml DAPI into 1 ml PBS
11. Aspirate off secondary antibody
12. Wash wells 5x with PBS-BSA
13. Wash wells 2x with PBS
14. Add 25 microliters of diluted DAPI
15. Incubate 5 min. at room temperature
16. Aspirate off DAPI
17. Wash 2x with PBS
18. Let slide air dry

Mounting

1. Rinse cover-slip with 95% EtOH and let air dry
2. Add a dollop of mounting media (Citifluor by Ted Pella) to slide
3. Put cover-slip on top and gently push the cover-slip to make sure mount covers the entire slide and that all air bubbles are expelled
4. Blot excess medium on paper towel
5. Seal edges with nail polish
6. When polish is dry, wash slide with water and dry with a kimwipe
7. Slides can be stored for several weeks at -20 degrees in the dark

4% paraformaldehyde solution

dissolve 2.72 g sucrose in 60 ml water

mix in 2 10 ml ampules 16% paraformaldehyde from Ted Pella (break open and remove liquid with needle attached to 10 ml syringe)

filter sterilize

store at 4 degrees

1 M KH₂PO₄

dissolve 68 g in 500 ml of warm water then autoclave

1 M K₂HPO₄

dissolve 87 g in 500 ml of water then autoclave

0.1 M KPO₄

41.7 ml 1 M K₂HPO₄

8.3 ml 1 M KH₂PO₄

450 ml water

filter sterilize

0.1 M KPO₄/1.2 M sorbitol

41.7 ml 1 M K₂HPO₄

8.3 ml 1 M KH₂PO₄

109.23 g sorbitol

water up to 500 ml

filter sterilize

PBS-BSA

5 g bovine serum albumin

5 ml 1 M KH₂PO₄

20 ml 1 M K₂HPO₄

15 ml 5 M NaCl

1 g NaN₃

dissolve and adjust volume to 500 ml

filter sterilize

store at 4 degrees