Yeast Immunofluorescence:

1. Grow approximately 10 mls of cells to log phase (O.D. ~ 0.5).

2. Fix cells by adding 1/10 volume of 37% formaldehyde to the culture. Incubate cells in formaldehyde for 2 hours. Some proteins may need short fixation time. If you are doing it for the first time, it is good to try several different fixation times. When staining for tubulin it is best not to fix for over 2 hrs.

3. Pellet the cells. Wash the cells twice with 1 volume of 1XPBS.

4. Resuspend cells in 0.5 ml spheroplasting buffer. This can be stored at 4^0C for 2-3 days.

5. Take 200 µl of cells and add 3.2 µl of 1.42M β-ME and 5 µl of 5 mg/ml zymolase 100T.

6. Incubate at 30^0C for 60 minutes. Check for sheroplasting under the microscope - cells that have no cell wall lose their shiny appearance. It takes 60 to 90 minutes to remove the cell walls of 90% of the cells.

7. Wash the cells once with 1 ml of PBS + 0.05% Tween 20 and resuspend in 100 µl of PBS + 0.05% Tween 20.

Staining

1. Prepare slides (10 well multiwell slides) by putting 50 µl of 1 mg/ml polylysine (size 400,00) onto each well. Let it sit for 15 mins. Aspirate off and let dry. Wash wells 3 times with water and let dry.

2. Put 20 μl of fixed cells onto each well. After approximately 5 min. aspirate off and wash 3 times with PBS.

3. Let slide dry for 5-10 minutes.

Optional Step. Immerse in methanol at -20C for 5 min. and then immerse in acetone at -20C for 30 seconds. Let it dry. This treatment results in flatter cells.

4. Put 20 μl of PBS + 1 mg / ml BSA onto each well. Incubate for 30 minutes in a humid chamber. Do not let the slides dry from this point on.

5. Aspirate off the blocking solution and wash 3 times with PBS.

6. Add 20 μl of diluted primary antibody (dilute in PBS + 1 mg / ml BSA). Incubate 1 hr in humid chamber at room temperature.

7. Aspirate off primary antibody and wash 3 times with PBS.

8. Add 20 μl of diluted secondary antibody and incubate in the dark.

9. Aspirate off secondary antibody and wash 3 times with PBS.

10. Add 20 μl of 1 μg/ml DAPI in PBS to each well.

11. Incubate approximately 2 minutes. Wash wells 3 times with PBS.

12. Add mounting solution to each well, and put on a coverslip. Blot away excess of mounting solution with a kimwipe. Seal the coverslip with nail polish and store the slides at -20^0C.