FM 4-64 labeling of yeast vacuole membranes

FM 4-64 is a lipophilic styryl dye sold by Molecular Probes (product no. T-3166; http://www.probes.com/). FM 4-64 is a vital stain: it fluoresces only in living cells, so cells cannot be fixed then stained nor stained then fixed. You must stain and observe living cells. FM 4-64 does not permeate cell membranes but, instead, intercalates into the plasma membrane is then taken into the cells by endocytosis (see Vida and Emr, J. Cell Biol. 1995 128:779-92).

1. Transfer 1 mL of log-phase cells (0.5-0.8 ODU/mL) to an eppi tube and centrifuge at 5000 x g for 5 min at room temperature (RT)

2. Aspirate supernatant; resuspend cell pellet in 50 μL YPD + 1 μL FM 4-64

   *FM 4-64 stock solution = 1.6 μM in DMSO (store at –20˚C)
   *FM 4-64 does not efficiently label cells in minimal medium, so even if you grew the cells in YNB in order to maintain a plasmid, you must label with FM in YPD

3. Incubate cells in a 30˚C water bath for 20 min

4. Add 1 mL YPD and centrifuge at 5000 x g for 5 min at RT

5. Aspirate supernatant; resuspend cell pellet in 1 mL YPD; transfer to culture tube

6. Add 4 mL YPD, then shake at 30˚C for 90-120 min

7. Transfer total volume (5 mL) to centrifuge tube and spin 5 min at 5000 x g at RT

8. Aspirate supernatant and resuspend cell pellet in 1 mL sterile water

9. Transfer volume to eppi tube and centrifuge at 5000 x g for 5 min at RT

10. Aspirate all of the supernatant and resuspend cell pellet in 25 μL YNB

    *YNB does not exhibit nearly as much autofluorescence as does YPD, so even if you grew the cells in YPD, resuspend the cells in YNB at this step
    *may need to adjust resuspension volume to optimize cell density for microscopy

11. Spot 7 μL of sample on ConA/polyK-coated glass slides and cover with an 18mm x 18mm cover slip (press coverslip to squeeze out air bubbles)

    *Coat glass slides with 10 μL of a 1:1 mixture of 2 mg/mL concanavalin A and 0.1% poly-L-lysine (spread mixture with side of pipet tip evenly over slide and let air dry); this mixture should effectively immobilize yeast cells on the glass slide due to the high amount of polysaccharides and negatively charged proteins in the cell wall

12. Observe fluorescence in microscope using Texas Red filter or Cy3 filter

    *The observed intensity of FM 4-64 fluorescence is greater using the Texas Red filter compared to the Cy3 filter (see filter spectra at http://www.chroma.com/)

Note: The above protocol is a pulse-chase procedure designed to label only the vacuole membranes of yeast cells with FM 4-64. You may, however, label the membranes of compartments in the entire endocytic pathway (plasma membrane, early and late endosomal membranes, and vacuole membranes) if you continuously pulse the cells with FM 4-64 for 60-120 min (i.e., do not chase in label-free medium). Conversely, you may label only the plasma membrane if you add FM 4-64 to cells on ice (of course, you will need to maintain the sample at 0˚C during microscopy, which could prove difficult.)

Also note: The above protocol is designed for cells that exhibit no temperature-sensitive mutations. If you want to observe a temperature-sensitive mutant, you will need to design your experiment accordingly.