

## Yeast Vacuole Staining with FM4-64

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**[Abstract]** The lipophilic probe, FM 4-64 does not fluoresce much in water but fluoresces strongly after binding to the outer plasma membrane, providing clear and distinguishable plasma membrane staining. The binding is rapid and reversible. In this protocol vacuoles in yeast cells are stained with the FM4-64 dye, permitting the use of live-cell imaging if needed.

### Materials and Reagents

1. FM4-64 (Life Technologies, Molecular Probes, catalog number: F34653)
2. DMSO (Sigma-Aldrich)
3. YES medium
4. FM4-64 stock solution
5. EMM
6. PBS

### Equipment

1. Water bath
2. Bench-top centrifuge

### Procedure

1. Grow yeast cells to exponential phase in YES medium at 30 °C.
2. Spin down cells and resuspend cell pellet in 500 µl YES + 0.5 µl FM4-64 stock solution (8 mM), so the final concentration of FM4-64 is 8 µM. Keep the cells in the dark (*i.e.*, wrapped in aluminum foil). Incubate cells in a 30 °C water bath for 30 min.  
 \*FM4-64 stock solution = 8 mM (5 µg/µl) in DMSO (stored at -20 °C).  
 \*FM4-64 does not efficiently label cells in minimal medium, so even if you grew the cells in EMM in order to maintain a plasmid, you must label cells with FM in YES.

3. Spin down cells and wash pellet by resuspending in YES to remove free FM4-64. Spin down again and resuspend in 1 ml YES. Transfer cells to a culture tube and add 4 ml YES and then shake at 30 °C for 90 min.
4. Transfer cells (5 ml) to centrifuge tube and spin 5 min at RT.
5. Resuspend cell pellet in EMM or PBS.  
EMM does not exhibit nearly as much autofluorescence as does YES, so even if you grew the cells in YES, resuspend the cells in EMM at this step.
6. Spot cells on a glass slide and cover with coverslip.
7. Observe fluorescence in microscope using RFP filter.

### **Notes**

This protocol is a pulse-chase procedure designed to label only the vacuole membranes of yeast cells with FM4-64. 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 FM4-64 for 60-120 min (*i.e.*, do not chase in label-free medium). Conversely, you may label only the plasma membrane if you add FM4-64 to cells on ice (of course, you will need to maintain the sample at 0 °C during microscopy, which could prove difficult).

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### **References**

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