Yeast Vacuole Staining with FM4-64

Zongtian Tong*

Department of Cell Biology, Center for Metabolism and Obesity Research, Johns Hopkins School of Medicine, Baltimore, USA

*For correspondence: tongzong@gmail.com

[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).

Acknowledgments

This protocol has been modified and adapted in the Espenshade Lab, Johns Hopkins School of Medicine. Funding to support different projects that have used this protocol has come from NIH – National Heart, Lung, and Blood Institute, National Institute of Allergy and Infectious Diseases, the Pancreatic Cancer Action Network, and the American Heart Association.

References

1. Rieder, S. E., Banta, L. M., Kohrer, K., McCaffery, J. M. and Emr, S. D. (1996). Multilamellar endosome-like compartment accumulates in the yeast vps28 vacuolar protein sorting mutant. *Mol Biol Cell* 7(6): 985-999.
2. Vida, T. A. and Emr, S. D. (1995). A new vital stain for visualizing vacuolar membrane dynamics and endocytosis in yeast. *J Cell Biol* 128(5): 779-792.