Probes for Yeast Vacuoles

Yeast Vacuole Marker Sampler Kit and Yeast Vacuole Membrane Marker MDY-64

Catalog Numbers Y7531, Y7536

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Product description

The Yeast Vacuole Marker Sampler Kit (Cat. No. Y7531) contains sample quantities of a series of both novel and well established vacuole marker probes that show promise for the study of yeast cell biology. Our recent experiments have demonstrated that several membrane-permeable chloromethyl coumarin derivatives are largely sequestered into yeast vacuoles. This kit includes samples of our CellTracker™ Blue CMAC and the aminopeptidase substrate CMAC-Ala-Pro.[1-3] Each of these coumarin-based vacuole markers contains a mildly reactive chloromethyl moiety that reacts with accessible thiols on peptides and proteins to form an aldehyde-fixable conjugate. The conjugate formed with CellTracker™ Blue CMAC is blue fluorescent, whereas the CMAC-Ala-Pro substrates require subsequent protease cleavage to activate their fluorescence. The probes selectively stain the lumen of the yeast vacuole. To complement the blue fluorescent staining of the lumen, we provide a new proprietary green fluorescent yeast vacuole membrane marker MDY-64 for staining the vacuole membrane. This kit also includes the commonly used vacuole marker 5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate (carboxy-DCFDA) as a standard.[4]

Contents and storage

Upon receipt, store the Yeast Vacuole Marker Sampler Kit and Yeast Vacuole Membrane Marker MDY-64 frozen at –20°C, desiccated and protected from light. When stored properly, both solids and resuspended stock solutions are stable for at least six months.

Component	Amount	Storage
carboxy-DCFDA (5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate) (Component A)	500 µg	–20°C
CellTracker™ Blue CMAC (7-amino-4-chloromethylcoumarin) (Component B)	500 µg	
CMAC-Ala-Pro (7-amino-4-chloromethylcoumarin, L-alanyl-L-proline amide) (Component C)	500 µg	
Yeast vacuole membrane marker MDY-64[1] (Component D)	200 µg	

[1] Yeast Vacuole Membrane Marker MDY-64 is also available separately as a solid in a unit size of 1 mg (Cat. No. Y7536).

Guidelines for resuspending reagents

- Allow the reagents to warm to room temperature before opening the vials.
- Dissolve kit Components A–D and Yeast Vacuole Membrane Marker MDY-64 in high-quality, anhydrous DMSO. See Table 1 for the required volume of solvent to add to each component of the kit to prepare a suitable stock solution.
- Divide stock solutions into aliquots and store frozen at –20°C, desiccated and protected from light.
- Avoid frequent freezing and thawing of reagents.

Table 1 Yeast Vacuole Marker Sampler Kit, preparation of stock solutions.

Stain	MW	Amount	Required volume of DMSO/vial	Stock concentration	Working concentrations
carboxy-DCFDA	529	500 µg	95 µL	10 mM	10 µM
CellTracker™ Blue CMAC	211	500 µg	237 µL	10 mM	100 µM
CMAC-Ala-Pro	414	500 µg	121 µL	10 mM	100 µM
Yeast vacuole membrane marker MDY-64	384	200 µg	52 µL	10 mM	10 µM

Staining protocol

The following protocols have been found to be simple and reliable for staining the vacuoles of *Saccharomyces cerevisiae*. These protocols may require modifications based on the particular cell type used and growth conditions used. Note that the green-fluorescent yeast vacuole membrane marker MDY-64 can be used in combination with either of the blue fluorescent lumen markers (CellTracker™ Blue CMAC or CMAC-Ala-Pro).

See Table 1 for the recommended working concentration for each of the components of the Yeast Vacuole Marker Sampler Kit.

Stain cells with carboxy-DCFDA

1. Resuspend cells at 10^6 cells/mL in 50 mM sodium citrate buffer, pH 5, containing 2% glucose.
2. Using the 10 mM DMSO stock solution, add carboxy-DCFDA (Component A) to a final concentration of 10 µM.
3. Incubate cells at room temperature for 15–30 minutes.
4. Visualize the stained cells by fluorescence microscopy (see Table 2 for spectral guidelines).

Stain cells with CMAC derivatives

1. Resuspend cells at 10^6 cells/mL in 10 mM HEPES buffer, pH 7.4, containing 5% glucose.
2. Using the 10 mM DMSO stock solution, add CellTracker™ Blue CMAC (Component B) or CMAC-Ala-Pro (Component C) to a final concentration of 100 µM.
3. Incubate the cells at room temperature for 15–30 minutes.
4. Visualize the stained cells by fluorescence microscopy (see Table 2 for spectral guidelines).

Stain cells with yeast vacuole membrane marker MDY-64

1. Resuspend cells at 10^6 cells/mL in 10 mM HEPES buffer, pH 7.4, containing 5% glucose.
2. Using the 10 mM DMSO stock solution, add yeast vacuole membrane marker MDY-64 (Component D) to a final concentration of 10 µM.
3. Incubate the cells at room temperature for ≤3–5 minutes.
4. Pellet the cells by centrifugation and resuspend them in fresh 10 mM HEPES buffer, pH 7.4, containing 5% glucose.
5. Visualize the stained cells by fluorescence microscopy (see Table 2 for spectral guidelines).

Spectral characteristics of yeast vacuole markers

Excitation and fluorescence emission maxima in nm for the fluorescent products.

Table 2

Stain	Ex	Em
carboxy-DCFDA[1]	504	529
CellTracker™ Blue CMAC	354	469
CMAC-Ala-Pro[2]	354	469
Yeast vacuole membrane marker MDY-64	451	497

[1] Carboxy-dichlorofluorescein diacetate is nonfluorescent until both the acetates are hydrolyzed.

[2] This substrate is essentially nonfluorescent until cleaved by intracellular peptidases.

References

1. Methods Enzymol 194, 428 (1991)
2. Arch Biochem Biophys 226, 292 (1983)
3. FEBS Lett 131, 296 (1981)
4. Methods Enzymol 194, 644 (1991)

Limited product warranty

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The information in this guide is subject to change without notice.