

## Probes for Yeast Vacuoles

**Y-7531 Yeast Vacuole Marker Sampler Kit**

**Y-7536 Yeast Vacuole Membrane Marker MDY-64**

### Quick Facts

#### Storage upon receipt:

- $-20^{\circ}\text{C}$
- Desiccate
- Protect from light

### Introduction

The Yeast Vacuole Marker Sampler Kit (Y-7531) 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 three samples of such reagents, including our CellTracker™ Blue CMAC and the aminopeptidase substrates CMAC-Arg and CMAC-Ala-Pro.<sup>1-3</sup> 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-Arg and CMAC-Ala-Pro substrates require subsequent protease cleavage to activate their fluorescence. All three 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.<sup>4</sup>

### Materials

#### Kit Contents

- carboxy-DCFDA (5-(and-6)-carboxy-2',7'-dichlorofluorescein diacetate) (Component A), 500  $\mu\text{g}$
- CellTracker Blue CMAC (7-amino-4-chloromethylcoumarin) (Component B), 500  $\mu\text{g}$
- CMAC-Arg (7-amino-4-chloromethylcoumarin, L-arginine amide) (Component C), 500  $\mu\text{g}$
- CMAC-Ala-Pro (7-amino-4-chloromethylcoumarin, L-alanyl-L-proline amide) (Component D), 500  $\mu\text{g}$
- yeast vacuole membrane marker MDY-64 (Component E), 200  $\mu\text{g}$

Table 1 shows the molecular weight (MW) of each component.

#### Yeast Vacuole Membrane Marker MDY-64

When purchased separately, yeast vacuole membrane marker MDY-64 (Y-7536) is supplied as a solid in a unit size of 1 mg.

#### Storage and Handling

Upon receipt, the Yeast Vacuole Marker Sampler Kit and yeast vacuole membrane marker MDY-64 should be stored frozen at  $-20^{\circ}\text{C}$ , desiccated and protected from light. Allow the reagents to warm to room temperature before opening the vials. Table 1 shows the volume of solvent to add to each component of the kit to prepare a suitable stock solution. Kit Components A–E and yeast vacuole membrane marker MDY-64 should be dissolved in high-quality, anhydrous DMSO. To avoid frequent freezing and thawing, divide stock solutions into aliquots and store frozen at  $-20^{\circ}\text{C}$ , desiccated and protected from light. When stored properly, both the solids and the stock solutions are stable for at least six months.

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

Stain	MW	Amount Supplied per Vial	Solvent	Volume of Solvent to Add per Vial	Stock Concentration	Working Concentrations
carboxy-DCFDA	529	500 $\mu\text{g}$	DMSO	95 $\mu\text{L}$	10 mM	10 $\mu\text{M}$
CellTracker Blue CMAC	211	500 $\mu\text{g}$	DMSO	237 $\mu\text{L}$	10 mM	100 $\mu\text{M}$
CMAC-Arg	439	500 $\mu\text{g}$	DMSO	114 $\mu\text{L}$	10 mM	100 $\mu\text{M}$
CMAC-Ala-Pro	414	500 $\mu\text{g}$	DMSO	121 $\mu\text{L}$	10 mM	100 $\mu\text{M}$
yeast vacuole membrane marker MDY-64	384	200 $\mu\text{g}$	DMSO	52 $\mu\text{L}$	10 mM	10 $\mu\text{M}$

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## Staining Protocol

Table 1 shows the recommended working concentration for each of the components of the Yeast Vacuole Marker Sampler Kit. 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 may be used in combination with any of the three blue fluorescent *lumen markers* (CellTracker Blue CMAC, CMAC-Arg or CMAC-Ala-Pro).

### Carboxy-DCFDA

**1.1** Resuspend cells at  $10^6$  cells/mL in 50 mM sodium citrate buffer, pH 5, containing 2% glucose.

**1.2** Using the 10 mM DMSO stock solution, add carboxy-DCFDA (Component A) to a final concentration of 10  $\mu$ M.

**1.3** Incubate cells at room temperature for 15–30 minutes.

**1.4** Visualize the stained cells by fluorescence microscopy (see *Fluorescence Microscopy*).

### CMAC Derivatives

**2.1** Resuspend cells at  $10^6$  cells/mL in 10 mM HEPES buffer, pH 7.4, containing 5% glucose.

**2.2** Using the 10 mM DMSO stock solution, add CellTracker Blue CMAC (Component B), CMAC-Arg (Component C) or CMAC-Ala-Pro (Component D) to a final concentration of 100  $\mu$ M.

**2.3** Incubate the cells at room temperature for 15–30 minutes.

**2.4** Visualize the stained cells by fluorescence microscopy (see Table 2 for spectral guidelines).

**Table 2.** Spectral characteristics of the yeast vacuole markers.

Stain	Ex *	Em *
carboxy-DCFDA ‡	504	529
CellTracker Blue CMAC	354	469
CMAC-Arg §	354	469
CMAC-Ala-Pro §	354	469
yeast vacuole membrane marker MDY-64	451	497

\* Excitation and fluorescence emission maxima in nm. † Carboxy-dichlorofluorescein diacetate is nonfluorescent until both the acetates are hydrolyzed. ‡ These substrates are essentially nonfluorescent until cleaved by peptidases; the table shows the excitation and emission maxima of the fluorescent products.

### Yeast Vacuole Membrane Marker MDY-64

**3.1** Resuspend cells at  $10^6$  cells/mL in 10 mM HEPES buffer, pH 7.4, containing 5% glucose.

**3.2** Using the 10 mM DMSO stock solution, add yeast vacuole membrane marker MDY-64 (Component E) to a final concentration of 10  $\mu$ M.

**3.3** Incubate the cells at room temperature for a maximum of 3–5 minutes.

**3.4** Pellet the cells by centrifugation and resuspend them in fresh 10 mM HEPES buffer, pH 7.4, containing 5% glucose.

**3.5** Visualize the stained cells by fluorescence microscopy (see *Fluorescence Microscopy*).

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## 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).

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## Product List

*Current prices may be obtained from our Web site or from our Customer Service Department.*

Cat #	Product Name	Unit Size
Y-7531	Yeast Vacuole Marker Sampler Kit .....	1 kit
Y-7536	yeast vacuole membrane marker MDY-64 .....	1 mg

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Further information on Molecular Probes' products, including product bibliographies, is available from your local distributor or directly from Molecular Probes. Customers in Europe, Africa and the Middle East should contact our office in Leiden, the Netherlands. All others should contact our Technical Assistance Department in Eugene, Oregon.

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### **Molecular Probes, Inc.**

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### **Molecular Probes Europe BV**

PoortGebouw, Rijnsburgerweg 10

2333 AA Leiden, The Netherlands

Phone: +31-71-5233378 ● Fax: +31-71-5233419

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