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Probes for Yeast Mitochondria

Y-7530 Yeast Mitochondrial Stain Sampler Kit
S-7529 SYTO® 18 yeast mitochondrial stain

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Ex/Em: See Table 2

Introduction

Molecular Probes' Yeast Mitochondrial Stain Sampler Kit (Y-7530) contains sample quantities of five different cell-permeant probes that have been found to label mitochondria in live yeast. In addition to the well characterized mitochondrial stains rhodamine 123[1] and $DiOC_6(3)$,[2,3] this kit includes rhodamine B hexyl ester, MitoTracker® Green FM and SYTO® 18 yeast mitochondrial stain, a proprietary green fluorescent nucleic acid stain. Each of these probes is sequestered in functioning mitochondria.

MitoTracker Green FM and SYTO 18 yeast mitochondrial stain represent two unique dyes from Molecular Probes. Like our other MitoTracker probes, MitoTracker Green FM accumulates in functioning mitochondria where its chloromethyl moiety reacts with accessible thiols on peptides and proteins to form an aldehyde-fixable green fluorescent conjugate. This property allows the sample to retain the fluorescent staining pattern characteristic of living cells during subsequent processing steps. Moreover, MitoTracker Green FM remains nonfluorescent in aqueous solution and becomes intensely fluorescent when it accumulates in a lipid environment. Thus, mitochondria stained with nanomolar concentrations of this probe exhibit bright green fluorescence with little or no background.

The SYTO 18 yeast mitochondrial stain is an effective mitochondrial stain in live yeast but does not penetrate higher eukaryotic cells. The SYTO 18 stain exhibits a pronounced fluorescence enhancement upon binding of mitochondrial nucleic acids, thereby providing very low background fluorescence even when cells are visualized in the presence of dye.

Each of the components of the Yeast Mitochondrial Stain Sampler Kit is also available separately, including MitoTracker Green FM (M-7514) and SYTO 18 yeast mitochondrial stain (S-7529). Table 1 shows the molecular weight (MW) of each component.

Materials

Kit Contents

- **Rhodamine 123** (Component A), 1 mg
- **Rhodamine B, hexyl ester, perchlorate** (Component B), 200 µg
- **MitoTracker Green FM** (Component C), 50 µg
- **SYTO 18 yeast mitochondrial stain** (Component D), 100 µg
- **$DiOC_6(3)$** (3,3′-dihexyloxacarbocyanine iodide), Component E, 1 mg

SYTO 18 Yeast Mitochondrial Stain

When purchased separately, SYTO 18 yeast mitochondrial stain (S-7529) is supplied as a 5 mM solution in dimethylsulfoxide (DMSO) in units of 250 µL.

Caution: No data are available addressing the mutagenicity or toxicity of SYTO 18 yeast mitochondrial stain. Because the reagent binds to nucleic acids, it should be treated as a potential

Table 1. *Preparation of stock solutions.*

Stain	MW	Amount supplied per vial	Solvent	Volume of solvent to add per vial	Stock Concentration	Working Concentrations
Rhodamine 123	381	1 mg	DMSO	105 µL	25 mM	30–50 µM
Rhodamine B, hexyl ester	627	200 µg	DMSO	319 µL	1 mM	100 nM
MitoTracker Green FM	672	50 µg	DMSO	74 µL	1 mM	100 nM
SYTO 18 yeast mitochondrial stain	723	100 µg	DMSO	28 µL	5 mM	10 µM
$DiOC_6(3)$	573	1 mg	ethanol	100 µL	17.5 mM	200 nM

mutagen and used with appropriate care. The DMSO stock solution should be handled with particular caution as DMSO is known to facilitate the entry of organic molecules into tissues. We strongly recommend using double gloves when handling the DMSO stock solution. As with all nucleic acid stains, solutions containing this reagent should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dyes.

Storage and Handling

Upon receipt, the Yeast Mitochondrial Stain Sampler Kit and the SYTO 18 stock solution should be stored frozen at -20°C, desiccated and protected from light. Allow reagents to warm to room temperature before opening vials. Table 1 shows the volume of solvent to add to each kit component to prepare a suitable stock solution. Kit components A–D should be dissolved in high-quality, anhydrous DMSO and kit component E should be dissolved in ethanol. To avoid frequent freezing and thawing, divide stock solutions into aliquots and store frozen at -20°C, desiccated and protected from light. When stored properly, both the solids and the stock solutions are stable for at least six months.

Staining Protocol

Table 1 shows the recommended working concentration for each of the components of the Yeast Mitochondrial Stain Sampler Kit. The following protocols have been found to be simple and reliable for staining the mitochondria of *Saccharomyces cerevisiae*. They may require modifications based on the particular cell type and growth conditions used.

Rhodamine 123

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

1.2 Using the 25 mM stock solution, add rhodamine 123 to a final concentration of 30–50 µM.

Table 2. *Special characteristics of the yeast mitochondrial stains.*

Stain	Ex (nm) *	Em (nm) †
rhodamine 123 ‡	505	534
rhodamine B, hexyl ester	555	579
MitoTracker Green FM ‡,§	490	516
SYTO 18 yeast mitochondrial stain **	468	533
$DiOC_6(3)$ ‡	484	501

***** Fluorescence excitation maximum. **†** Fluorescence emission maximum. **‡** Excitation and emission maxima were determined in methanol; values may vary somewhat in cellular environments. **§** This probe is essentially nonfluorescent in aqueous solution. ****** This probe is essentially nonfluorescent until bound to DNA; the table shows the excitation and emission maxima of the DNA/dye complex.

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

1.4 Visualize by fluorescence microscopy.

Rhodamine B, Hexyl Ester

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

2.2 Using the 1 mM stock solution, add rhodamine B, hexyl ester to a final concentration of 100 nM.

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

2.4 Visualize by fluorescence microscopy.

MitoTracker Green FM

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

3.2 Using the 1 mM stock solution, add MitoTracker Green FM to a final concentration of 100 nM.

3.3 Incubate cells at room temperature for 15–30 minutes.

3.4 Visualize by fluorescence microscopy.

SYTO 18 Yeast Mitochondrial Stain

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

4.2 Using the 5 mM stock solution, add SYTO 18 yeast mitochondrial stain to a final concentration of 10 µM.

4.3 Incubate cells at room temperature for 3–5 minutes.

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

4.5 Visualize by fluorescence microscopy.

$DiOC_6(3)$ (see reference 2)

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

5.2 Dilute the 17.5 mM stock solution of $DiOC_6(3)$ 1:1000 in ethanol.

5.3 Using the diluted stock solution (17.5 µM), add $DiOC_6(3)$ to a final concentration of 175 nM.

5.3 Incubate cells at room temperature for 15–30 minutes.

5.4 Visualize by fluorescence microscopy.

References

1. Curr Genet 18, 265 (1990); **2.** Cell Motil Cytoskeleton 25, 111 (1993); **3.** Biochem Int 2, 503 (1981).

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

Cat #	Product Name	Unit Size
D-273	3,3′-dihexyloxacarbocyanine iodide ($DiOC_6(3)$)	100 mg
M-7514	MitoTracker® Green FM *special packaging*	20x50 µg
R-302	rhodamine 123	25 mg
R-648	rhodamine B, hexyl ester, chloride (R 6)	10 mg
S-7529	SYTO® 18 yeast mitochondrial stain *5 mM solution in DMSO*	250 µL
Y-7530	Yeast Mitochondrial Stain Sampler Kit	1 kit

Contact Information

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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