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SYTO® Red Fluorescent Nucleic Acid Stains

Quick Facts

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

Abs/Em: See Table 1

Note: Handle DMSO stock solutions with care.

Introduction

SYTO® dyes are cell-permeant nucleic acid stains that differ from each other in one or more characteristics, including cell permeability, fluorescence enhancement upon binding nucleic acids, excitation and emission spectra, DNA/RNA selectivity and binding affinity. Thus, each SYTO stain is potentially useful for a different range of applications. The SYTO dyes do not act exclusively as nuclear stains in live cells and should not be equated with DNA-selective compounds such as Hoechst 33258 (H-1398, H-3569) or Hoechst 33342 (H-1399, H-3570) that readily stain nuclei in animal cells. Eukaryotic cells incubated with SYTO dyes generally show cytoplasmic or mitochondrial staining as well as nuclear staining. Mitochondrial staining predominates in yeast and animal cells stained with SYTO 59–64 stains. In addition, SYTO dyes will stain most live and permeabilized bacteria. The SYTO red fluorescent stains are available individually as well as in the sampler kit (S-11340). The sampler kit helps researchers develop the optimal combination of dyes, concentrations and protocols for their particular application. We suggest only broad ranges of staining concentrations, based on our laboratory experience, in order to provide a starting point for experiments. These conditions will require adjustment for each experimental system.

Even though all of the stains exhibit excitation *maxima* ~600 nm and above, they are easily visualized in the epifluorescence microscope using standard Texas Red® optical filter sets. In addition, the UV absorption of SYTO 59 stain (S-11341) allows it to be used in a dual-emission combination with other UV-excitable stains, such as SYTOX® Green nucleic acid stain (S-7020).[1] SYTO 59 has been used with SYTOX Green stain to image live and dead bacteria in the wide-field epifluorescence and laser confocal scanning microscopes.[1] SYTO 59 has also been used to evaluate the viability of *Cryptosporidium* oocysts.[2] SYTO 17 has been used to assess the effects of cytotoxic agents on *Escherichia coli* by flow cytometry.[3]

Materials

The red fluorescent SYTO dyes (SYTO 17 and SYTO 59–64) are each supplied as solutions in dimethylsulfoxide (DMSO) at a concentration of 5 mM. Individually packaged dyes are provided in unit sizes of 250 µL, except for SYTO 59 and SYTO 64, which have unit sizes of 100 µL. The SYTO Red Fluorescent Stain Sampler Kit (S-11340) contains 50 µL samples of each dye. Upon receipt, these vials should be stored frozen at -20°C, upright and protected from light. *Before opening, the vials should be allowed to warm to room temperature and then briefly centrifuged in a microcentrifuge to deposit the DMSO solution at the bottom of the vial.* Before refreezing, seal all vials tightly. When stored properly, these stock solutions are stable for at least one year.

Caution: No data are available addressing the mutagenicity or toxicity of these reagents. Because the reagents bind to nucleic acids, they should be treated as potential mutagens and used with appropriate care. The DMSO stock solutions 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 DMSO stock solutions. As with all nucleic acid stains, solutions containing these reagents should be poured through activated charcoal before disposal. The charcoal must then be incinerated to destroy the dyes.

Spectral Characteristics

Table 1 summarizes the absorption and emission maxima for the red fluorescent SYTO dyes. Because of the large fluorescence enhancement upon binding nucleic acids, absorption and emission maxima were determined for SYTO dyes in the presence of DNA. The full spectra of each SYTO Red dye bound to DNA is shown in Molecular Probes' document *Spectra for Red Fluorescent SYTO® Dyes* (TD 11341), available from our web site at www.probes.com/media/pis/td11341.pdf.

Experimental Guidelines

The following procedure can be adapted for most cell types. Note that different concentration ranges for the SYTO dyes are suggested depending on the cell type. Growth medium, cell density, the presence of other cell types and other factors may influence staining. In general, the best results are obtained in buffers that do not contain phosphate. Residual detergent on glassware may also affect real or apparent staining of many organisms, causing brightly stained material to appear in solutions with or without cells present. Glassware should be washed in a mild

Table 1. *Spectral characteristics of SYTO 17 and SYTO 59–SYTO 64 red fluorescent nucleic acid stains.*

Dye	Cat #	Abs (nm)*	Em (nm)*	QY† DNA
SYTO 17	S-7579	621	634	0.21
SYTO 59	S-11341	622	645	0.18
SYTO 60	S-11342	652	678	0.16
SYTO 61	S-11343	628	645	0.18
SYTO 62	S-11344	652	676	0.27
SYTO 63	S-11345	657	673	0.17
SYTO 64	S-11346	599	619	0.39

* Absorption and fluorescence emission maxima determined in the presence of DNA using a ratio of ~100 base pairs of nucleic acid to 1 dye molecule. The spectra were acquired in 50 mM Tris, 1 mM EDTA, pH 7.5.
† The fluorescence quantum yield was determined for SYTO 17 and SYTO 59–64 in the presence of DNA and expressed relative to that determined for cresyl violet in methanol.

detergent and rinsed with hot tap water followed by several rinses with deionized, distilled water.

Pellet cells by centrifugation and resuspend in buffered salt solution or water. Adherent cells in culture may be stained *in situ* on coverslips. Add SYTO stain(s) using the concentrations listed in Table 2 as a guide. In initial experiments, it may be best to try several dye concentrations over the entire suggested range to determine the concentration that yields optimal staining.

Table 2. *Recommended conditions for staining cells with red fluorescent SYTO dyes.*

Cell Type	SYTO Dye Concentration	Incubation Conditions
Bacteria	50 nM–20 µM	Vortex to mix, then incubate for 1–30 minutes
Yeast	1 µM–100 µM	Vortex to mix, then incubate for 10–120 minutes
Animal cells	10 nM–5 µM	Incubate for 10–120 minutes

Cells stained with SYTO 17 and SYTO 59–64 red fluorescent nucleic acid stains can be visualized with a Texas Red filter set.

Stained eukaryotic cells will generally show diffuse cytoplasmic staining as well as nuclear staining. Fluorescent staining of intranuclear bodies is frequently observed. Because these dyes are cell-permeant and contain a net positive charge at neutral pH, they typically stain mitochondria. Staining of live yeast is also primarily mitochondrial.

References

1. B.L. Roth, et al., ASM Annual Meeting abstract I-23 (1997); **2.** Intl J Parasitol 27, 787 (1997); **3.** Cytometry 29, 58 (1997).

Product List

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

Cat #	Product Name	Unit Size
S-7579	SYTO® 17 red fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-11341	SYTO® 59 red fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL
S-11342	SYTO® 60 red fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-11343	SYTO® 61 red fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-11344	SYTO® 62 red fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-11345	SYTO® 63 red fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-11346	SYTO® 64 red fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL
S-11340	SYTO® Red Fluorescent Stain Sampler Kit *SYTO® dyes 17 and 59-64* *50 µL each*	1 kit

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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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