

SYTO[®] Orange Fluorescent Nucleic Acid Stains

Quick Facts

Storage upon receipt:

- -20°C
- Desiccate
- Protect from light

Abs/Em: See Table 1

Introduction

Molecular Probes' 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 or Hoechst 33342 that readily stain nuclei in animal cells. Eukaryotic cells incubated with SYTO dyes generally show cytoplasmic or mitochondrial staining as well as nuclear staining. In addition, SYTO dyes will stain most live and permeabilized bacteria.

With the introduction of the SYTO orange dyes, the SYTO series of dyes now spans the entire visible spectrum. The SYTO orange fluorescent stains, with optimal excitation wavelengths in the 530 nm to 570 nm range, are available individually as well as in a sampler kit (S-11360). 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 experi-

Table 1. Spectral characteristics of SYTO orange-fluorescent nucleic acid stains.*

Dye	Cat #	Abs (nm)	Em (nm)
SYTO 80	S-11361	531	545
SYTO 81	S-11362	530	544
SYTO 82	S-11363	541	560
SYTO 83	S-11364	543	559
SYTO 84	S-11365	567	582
SYTO 85	S-11366	567	583

* Absorption and fluorescence emission maxima determined in the presence of DNA in 10 mM Tris, 1 mM EDTA, pH 8.0.

ence, in order to provide a starting point for experiments. These conditions will require adjustment for each experimental system.

Contents, Storage and Handling

The orange fluorescent SYTO dyes 80–85 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. The SYTO Orange Fluorescent Stain Sampler Kit (S-11360) contains 50 µL samples of each dye. Upon receipt, these vials should be stored frozen at -20°C, upright, desiccated 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 SYTO orange fluorescent nucleic acid stains. Because of the large fluorescence enhancement upon binding nucleic acids, absorption and emission maxima were determined for SYTO dyes in the presence of DNA.

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. Staining can be affected by the growth medium used, the cell density, the presence of other cell types and other factors. 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 detergent and rinsed with hot tap water followed by several rinses with deionized water.

Pellet cells by centrifugation and resuspend in buffered salt solution. 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.

Cells stained with SYTO orange fluorescent nucleic acid stains can be viewed with a microscope equipped with a filter set appropriate for tetramethylrhodamine.

Stained eukaryotic cells will generally show diffuse cytoplasmic staining as well as nuclear staining. Fluorescent staining of intranuclear bodies is frequently observed. Also because these dyes are cell-permeant and contain a net positive charge at neutral pH, they occasionally stain mitochondria.

Table 2. Recommended conditions for SYTO orange-fluorescent nucleic acid stains.

Cell Type	SYTO Dye Concentration	Incubation Conditions
Bacteria	0.1–10 μ M	Vortex to mix, then incubate for 1–30 minutes
Yeast	0.5–50 μ M	Vortex to mix, then incubate for 10–120 minutes
Animal cells	0.1–5 μ M	Incubate for 10–120 minutes

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

Cat #	Product Name	Unit Size
S-11361	SYTO [®] 80 orange fluorescent nucleic acid stain *5 mM solution in DMSO*	250 μ L
S-11362	SYTO [®] 81 orange fluorescent nucleic acid stain *5 mM solution in DMSO*	250 μ L
S-11363	SYTO [®] 82 orange fluorescent nucleic acid stain *5 mM solution in DMSO*	250 μ L
S-11364	SYTO [®] 83 orange fluorescent nucleic acid stain *5 mM solution in DMSO*	250 μ L
S-11365	SYTO [®] 84 orange fluorescent nucleic acid stain *5 mM solution in DMSO*	250 μ L
S-11366	SYTO [®] 85 orange fluorescent nucleic acid stain *5 mM solution in DMSO*	250 μ L
S-11360	SYTO [®] Orange Fluorescent Stain Sampler Kit *SYTO [®] dyes 80-85* *50 μ L each*	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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