SYTO® Green-Fluorescent Nucleic Acid Stains

Quick Facts

Storage upon receipt:

- ≤–20ºC
- Protect from light

Abs/Em: See Table 1

Notes: Handle DMSO stock solutions with care.

SYTO® dyes are cell-permeant nucleic acid stains that show a large fluorescence enhancement upon binding nucleic acids. The SYTO dyes can be used to stain RNA and DNA in both live and dead eukaryotic cells, as well as in Gram-positive and Gram-negative bacteria. Available as blue-, green-, orange- or red-fluorescent dyes, these novel SYTO stains share several important characteristics:

- Permeability to virtually all cell membranes, including mammalian cells and bacteria
- High molar absorptivity, with extinction coefficients >50,000 $cm^{-1}M^{-1}$ at visible absorption maxima
- Extremely low intrinsic fluorescence, with quantum yields typically <0.01 when not bound to nucleic acids
- Quantum yields that are typically >0.4 when bound to nucleic acids

SYTO dyes 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. The SYTO dyes are compatible with a variety of fluorescence-based instruments that use either laser excitation or a conventional broadband illumination source (e.g., mercury- and xenon-arc lamps). SYTO nucleic acid stains have been used in diverse applications from staining DNA spotted on microarrays[1] to staining live and fixed cells. The SYTO dyes do not act exclusively as nuclear stains in live cells and should not be equated with DNA-selective compounds such as DAPI (D-1306, D-21490) or Hoechst 33342 (H-1399, H-3570), which stain nuclei in live animal cells. Eukaryotic cells incubated

Table 1. Spectral characteristics of Molecular Probes' SYTO green-fluorescent nucleic acid stains.

Dye	Cat #	Absorption * (nm)		Emission * (nm)		QY † DNA	QY † RNA
		+DNA	+RNA	+DNA	+RNA		
SYTO 9	S-34854	485	486	498	501	0.58	ND
SYTO 11	S-7573	508	510	527	530	0.49	0.39
SYTO 12	S-7574	499	500	522	519	0.09	0.13
SYTO 13	S-7575	488	491	509	514	0.40	0.40
SYTO 14	S-7576	517	521	549	547	0.08	0.12
SYTO 15	S-7577	516	518	546	555	0.15	0.20
SYTO 16	S-7578	488	494	518	525	0.65	0.24
SYTO 18	S-7529	490	493	507	527	0.24	0.31
SYTO 20	S-7555	512	ND	530	ND	0.16	ND
SYTO 21	S-7556	494	ND	517	ND	~0.5	ND
SYTO 22	S-7557	515	ND	535	ND	ND	ND
SYTO 23	S-7558	499	ND	520	ND	ND	ND
SYTO 24	S-7559	490	ND	515	ND	0.76	ND
SYTO 25	S-7560	521	ND	556	ND	ND	ND
SYTO BC	S-34855	485	487	500	504	ND	ND

***** Absorption and fluorescence emission maxima determined in the presence of DNA or RNA using a ratio of ~100 bases of nucleic acid to 1 dye molecule. For SYTO 11–18 dyes plus DNA the spectra were determined in 10 mM Tris, 1 mM EDTA, 50 mM NaCl, pH 7.4; for SYTO 11–18 dyes plus RNA the spectra were determined in 25 mM HEPES, pH 7.5; for SYTO 20–25 the spectra were determined in 50 mM Tris, 1 mM EDTA, pH 7.5. **†** The fluorescence quantum yield was determined for SYTO dyes in the presence of DNA or RNA and expressed relative to that determined for fluorescein in buffer at pH 9.0 (assumed to be 0.92 under these conditions). ND = not determined.

Table 2. Suggested conditions for staining with SYTO green-fluorescent nucleic acid stains.

Application	SYTO Dye Concentration	Staining Conditions
Bacterial cells	50 nM–20 µM	Vortex to mix, then incubate for 1–30 minutes
Eukaryotic cells	10 nM–5 µM	Incubate for 10–120 minutes
Microarrays	50 nM in TE buffer	Incubate for 5 minutes, rinse and then dry

with SYTO dyes generally show cytoplasmic or mitochondrial staining as well as nuclear staining.

The SYTO green-fluorescent nucleic acid stains have proven valuable in a broad range of research applications. SYTO 9 stain (S-34854) has been shown to stain live and dead Gram-positive and Gram-negative bacteria, and it is a component of the LIVE/DEAD *Bac*Light Bacterial Viability Kits (L-7007, L-7012, L-13152). SYTO 11 stain (S-7573) has been used in conjunction with time-lapse microscopy to examine the cleavage orientation of dividing cells in developing cerebral cortex.[2] SYTO 14 stain (S-7576) binds to cytoplasmic RNA, allowing its use in tracking RNA granule transport in living neurons.[3] A combination of propidium iodide (P-1304, P-3566) and SYTO 13 stain (S-7575) has allowed researchers to monitor glutamate-induced necrosis in cerebellar granule cells.[4] Several reports describe the use of SYTO dyes for detecting apoptosis.[5,6] A series of SYTO nucleic acid stains was screened for the ability to discriminate between apoptotic and non-apoptotic mouse thymocytes, and SYTO 16 stain (S-7578) was found to be optimal for this application.[7] SYTO 16 stain has also been used with propidium iodide to differentiate live and dead COS-7 cells with a laser-based scanning cytometer.[8] SYTO 18 stain (S-7529) is especially useful as a stain for yeast mitochondria (Molecular Probes, unpublished data). SYTO BC is a mixture of the best SYTO dyes for bacterial staining and is a component of the Bacteria Counting Kit (B-7277).

The green-fluorescent SYTO nucleic acid stains are available individually (Table 1), as well as in two different sampler kits. The sampler kits are useful for determining the optimal SYTO dye for a particular application.

Materials

Contents

- **SYTO dyes 11–15, 18 and 21–25**, 5 mM solutions in dimethylsulfoxide (DMSO), 250 µL
  SYTO 18 dye is also featured in our Yeast Mitochondrial Stain Sampler Kit (Y-7530).
- **SYTO 9 dye**, a 5 mM solution in DMSO, 100 µL
  SYTO 9 dye is a component of the LIVE/DEAD BacLight Bacterial Viability Kits (L-7007, L-7012, L13152).
- **SYTO 16 and SYTO 20 dyes**, 1 mM solutions in DMSO, 250 µL
- **SYTO BC dye mixture**, a 5 mM solution (total dye concentration) in DMSO, 100 µL
  The SYTO BC mixture is the staining component in the Bacteria Counting Kit (B-7277).
- **SYTO Green Fluorescent Nucleic Acid Stain Sampler Kits**
  Kit #1, S-7572, SYTO dyes 11–16, 50 µL samples of six different dyes
  Kit #2, S-7554, SYTO dyes 20–25, 50 µL samples of six different dyes

Storage and Handling

Upon receipt, store the vials of dye frozen at ≤–20°C, upright and protected from light. *Before opening, allow the vials to warm to room temperature and then briefly centrifuge in a microcentrifuge to bring the DMSO solution to 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.

Spectral Characteristics

When bound to nucleic acids, SYTO green-fluorescent nucleic acid stains have excitation and emission spectra similar to those of fluorescein (FITC) and can be visualized using optical filters appropriate for fluorescein. The full spectra of each SYTO green-fluorescent dye bound to nucleic acid is shown in Molecular Probes' document *Spectra for Green-Fluorescent SYTO® Dyes* (TD 07573); this document can be found at our Web site, linked directly from the Product Page of any of the green-fluorescent SYTO dyes mentioned in this Product Information Sheet.

Experimental Guidelines

In the guidelines below, we suggest broad ranges of staining concentrations, based on our laboratory experience or methods used in published papers, in order to provide a starting point for experiments. These conditions will require adjustment for each cell type and experimental system.

Use plastic tubes when diluting any SYTO stain, as the diluted stain will readily adhere to glass. In general, the best results are obtained in buffers that do not contain phosphate. When preparing other solutions, note that residual detergent on plastic or glassware may also affect real or apparent staining of many cells or organisms, causing brightly stained material to appear in solutions with or without cells present. All labware should be washed in a mild detergent and rinsed with hot tap water followed by several rinses with deionized water.

Adherent cells in culture may be stained *in situ* on coverslips. Cells in suspension should be pelleted by centrifugation and resuspended in buffered salt solution or water. Add the 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. Be aware that growth medium, cell density, the presence of other cell types and other factors may influence staining. Stained eukaryotic cells will generally show diffuse cytoplasmic staining as well as nuclear

staining. Particularly intense staining of intranuclear bodies is frequently observed. Because these dyes are cell permeant and contain a net positive charge at neutral pH, they may also stain mitochondria. Staining of live yeast is primarily mitochondrial.

SYTO dyes have proven to be useful for staining DNA on microarrays for quality control purposes. The staining conditions listed on Table 2 are adapted from a published experiment using SYTO 61 red fluorescent nucleic acid stain[1] (S-11343) and should be generally applicable for staining with other SYTO dyes.

References

1. Nucl Acids Res 29, e41 (2001); **2.** Cell 82, 631 (1995); **3.** J Neuroscience 16, 7812 (1996); **4.** Neuron 15, 961 (1995); **5.** Mol Biol Cell 6, 444a, abstract 1805 (1995); **6.** Nature 377, 20 (1995); **7.** Cytometry 21, 265 (1995); **8.** Cytometry 23, 272 (1996).

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

Cat #	Product Name	Unit Size
S-34854	SYTO® 9 green fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL
S-7573	SYTO® 11 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7574	SYTO® 12 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7575	SYTO® 13 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7576	SYTO® 14 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7577	SYTO® 15 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7578	SYTO® 16 green fluorescent nucleic acid stain *1 mM solution in DMSO*	250 µL
S-7529	SYTO® 18 yeast mitochondrial stain *5 mM solution in DMSO*	250 µL
S-7555	SYTO® 20 green fluorescent nucleic acid stain *1 mM solution in DMSO*	250 µL
S-7556	SYTO® 21 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7557	SYTO® 22 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7558	SYTO® 23 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7559	SYTO® 24 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-7560	SYTO® 25 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S-34855	SYTO® BC green fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL
S-7572	SYTO® Green Fluorescent Nucleic Acid Stain Sampler Kit #1 *SYTO® dyes 11-16* *50 µL each*	1 kit
S-7554	SYTO® Green Fluorescent Nucleic Acid Stain Sampler Kit #2 *SYTO® dyes 20-25* *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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