

SYTO® Green-Fluorescent Nucleic Acid Stains

Table 1 Contents and storage

Material	Amount	Concentration	Storage	Stability
SYTO® 9	100 µL	5 mM solution in DMSO	<ul style="list-style-type: none"> • ≤ -20°C • Protect from light 	When stored as directed, the product is stable for 1 year
SYTO® 11, 13, 14, 21, 24	250 µL	5 mM solution in DMSO		
SYTO® 12	315 µL	4 mM solution in DMSO		
SYTO® 16	250 µL	1 mM solution in DMSO		
SYTO® BC dye mixture	100 µL	5 mM solution (total dye concentration) in DMSO		
SYTO® Green-Fluorescent Nucleic Acid Stain Sampler Kit	1 kit (50 µL samples of 8 dyes)	Includes SYTO® dyes 11-14, 16, 21, 24		
Approximate fluorescence excitation/emission maxima: See Table 2 (page 3).				

Introduction

SYTO® green-fluorescent nucleic acid stains 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

For Research Use Only. Not for use in diagnostic procedures.

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 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 microarrays¹ 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 (Cat. nos. D1306, D21490) or Hoechst[®] 33342 (Cat. nos. H1399, H3570), which stain nuclei in live animal cells. Eukaryotic cells incubated 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 (Cat. no. S34854) has been shown to stain live and dead Gram-positive and Gram-negative bacteria, and is a component of the LIVE/DEAD *BacLight* Bacterial Viability Kits (Cat. nos. L7007, L7012, L13152). SYTO[®] 11 stain (Cat. no. S7573) 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 (Cat. no. S7576) binds to cytoplasmic RNA, allowing its use in tracking RNA granule transport in living neurons.³ A combination of propidium iodide (Cat. nos. P1304, P3566) and SYTO[®] 13 stain (Cat. no. S7575) 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 (Cat. no. S7578) 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[®] BC is a mixture of the best SYTO[®] dyes for bacterial staining and is a component of the Bacteria Counting Kit (Cat. no. B7277).

The SYTO[®] green-fluorescent nucleic acid stains are available individually (Table 2), as well as in a sampler kit. The sampler kit is useful for determining the optimal SYTO[®] dye for a particular application.

Table 2 Spectral characteristics of SYTO® green-fluorescent nucleic acid stains

Dye	Cat. no.	Absorption* (nm)		Emission* (nm)		QY [†] DNA	QY [†] RNA
		+DNA	+RNA	+DNA	+RNA		
SYTO® 9	S34854	485	486	498	501	0.58	ND
SYTO® 11	S7573	508	510	527	530	0.49	0.39
SYTO® 12	S7574	499	500	522	519	0.09	0.13
SYTO® 13	S7575	488	491	509	514	0.40	0.40
SYTO® 14	S7576	517	521	549	547	0.08	0.12
SYTO® 16	S7578	488	494	518	525	0.65	0.24
SYTO® 21	S7556	494	ND	517	ND	~0.5	ND
SYTO® 24	S7559	490	ND	515	ND	0.76	ND
SYTO® BC	S34855	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® 11–16 dyes plus DNA the spectra were determined in 10 mM Tris, 1 mM EDTA, 50 mM NaCl, pH 7.4; for SYTO® 11–16 dyes plus RNA the spectra were determined in 25 mM HEPES, pH 7.5; for SYTO® 21 and 24 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.

Before starting

Storage and handling Upon receipt, store the vials of dye frozen at $\leq -20^{\circ}\text{C}$, upright, and protected from light. Before opening, allow the vials to warm to room temperature and then briefly centrifuge 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 1 year.

Caution No data are available addressing the mutagenicity or toxicity of these reagents. The reagents bind to nucleic acids, so treat the dyes as potential mutagens and use with appropriate care. Handle the stock solutions with caution, because 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 the Appendix (Figures 1–4, pages 5–7).

Experimental guidelines

We suggest broad ranges of staining concentrations, based on our laboratory experience or published methods, to provide a starting point for experiments. These conditions require adjustment for each cell type and experimental system.

Use plastic tubes when diluting any SYTO® stain, because the diluted stain adheres 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. Wash all labware in mild detergent and rinse with hot tap water followed by several rinses with deionized water.

Adherent cells in culture may be stained *in situ* on coverslips. Pellet cells in suspension by centrifugation and resuspend in buffered salt solution or water. Add the SYTO® stain(s) using the concentrations listed in Table 3 (page 4) 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 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 3 (page 4) are adapted from a published experiment using SYTO® 61 red fluorescent nucleic acid stain¹ (Cat. no. S11343) and applicable for staining with other SYTO® dyes.

Table 3 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.

References

1. Nuc 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].

Appendix

Figure 1 Spectra of SYTO® 9 and SYTO® BC Green-Fluorescent Nucleic Acid Stains

A) Effect of DNA on absorption spectra. B) Effect of RNA on absorption spectra. C) Fluorescence emission spectra when bound to DNA or RNA.

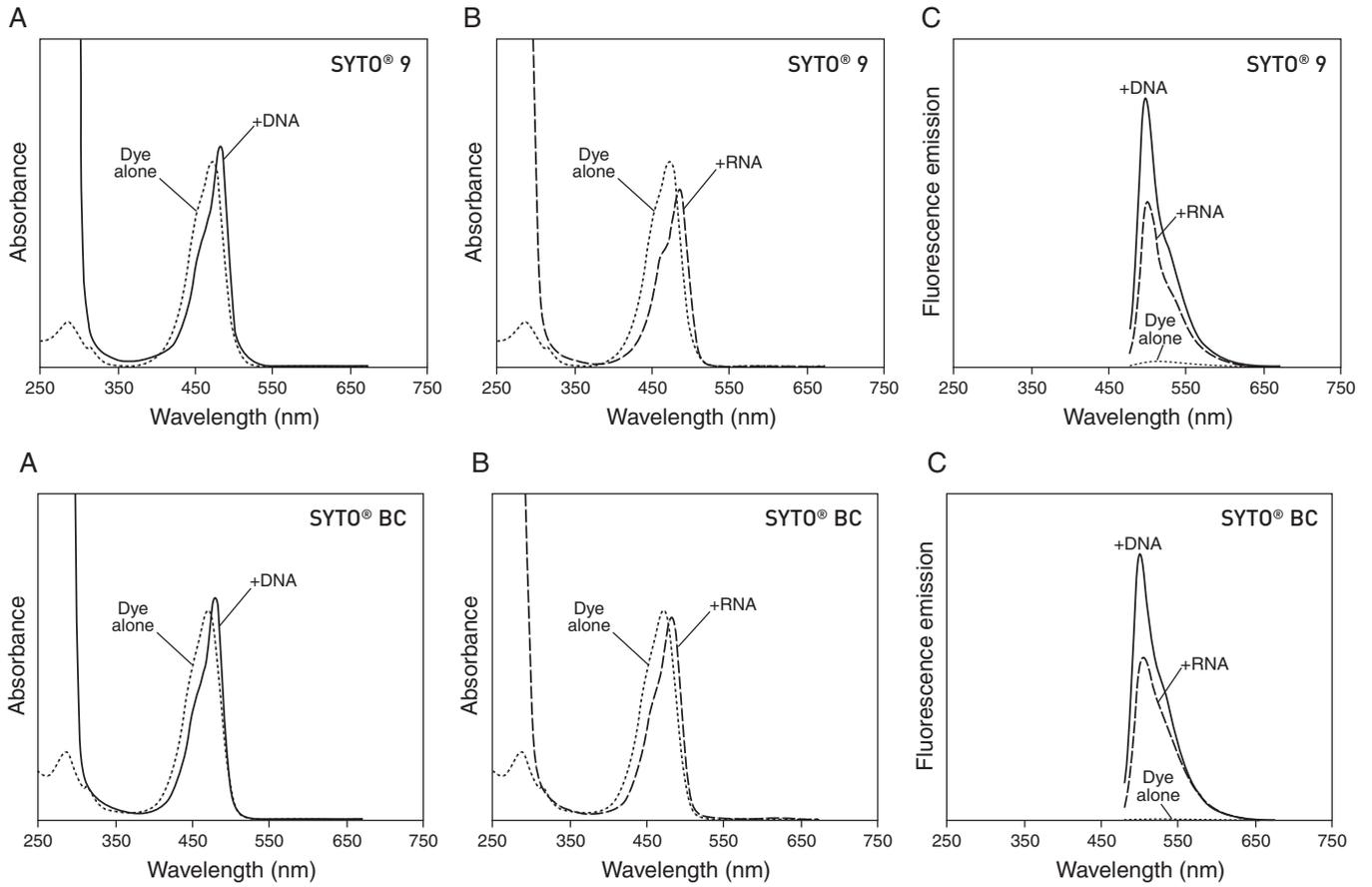


Figure 2 Spectra of SYTO® 11, 12, 13, and 14 Green-Fluorescent Nucleic Acid Stains

A) Effect of DNA on absorption spectra. B) Effect of RNA on absorption spectra. C) Fluorescence emission spectra when bound to DNA or to RNA.

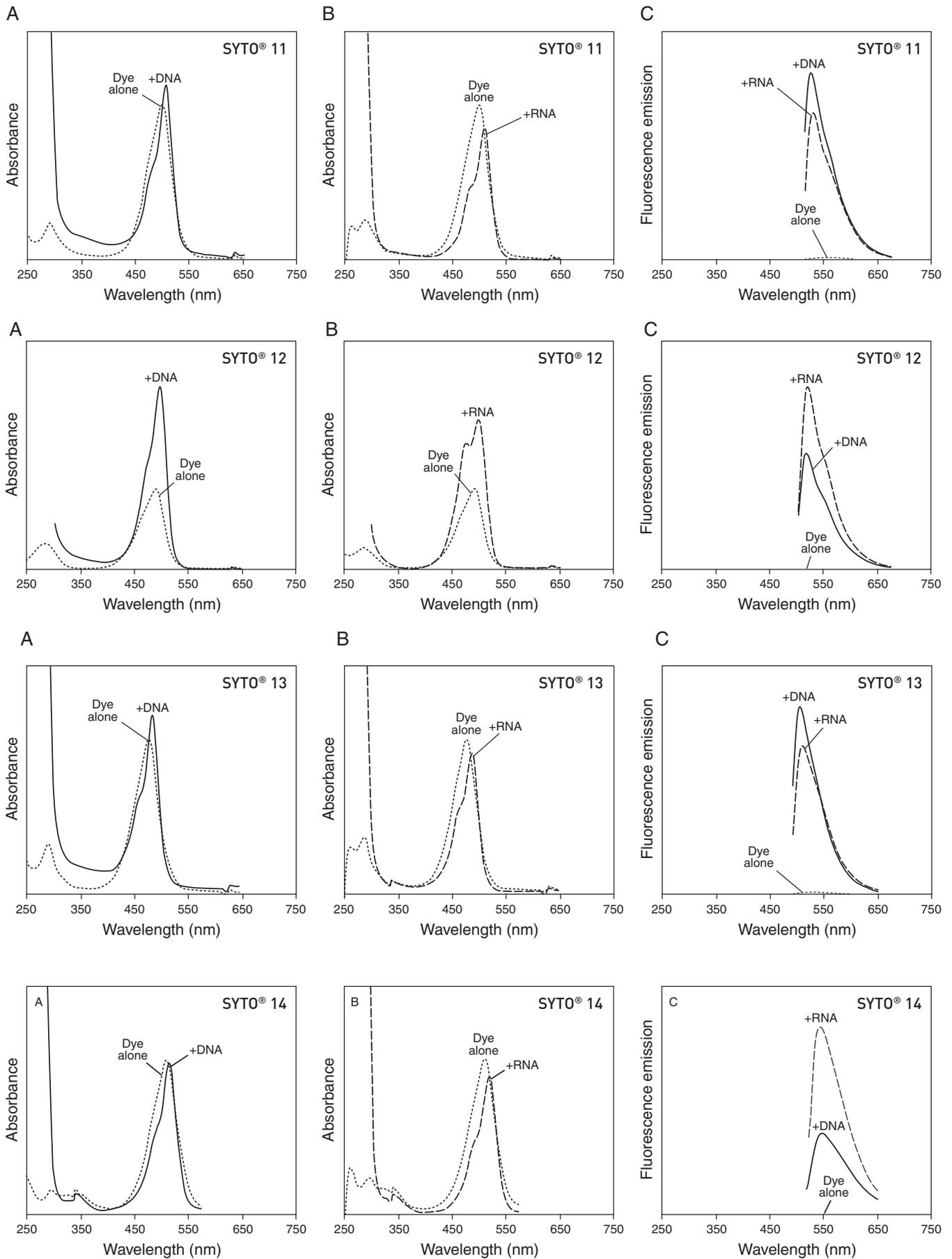


Figure 3 Spectra of SYTO® 16 Green-Fluorescent Nucleic Acid Stains

A) Effect of DNA on absorption spectra. B) Effect of RNA on absorption spectra. C) Fluorescence emission spectra when bound to DNA or to RNA.

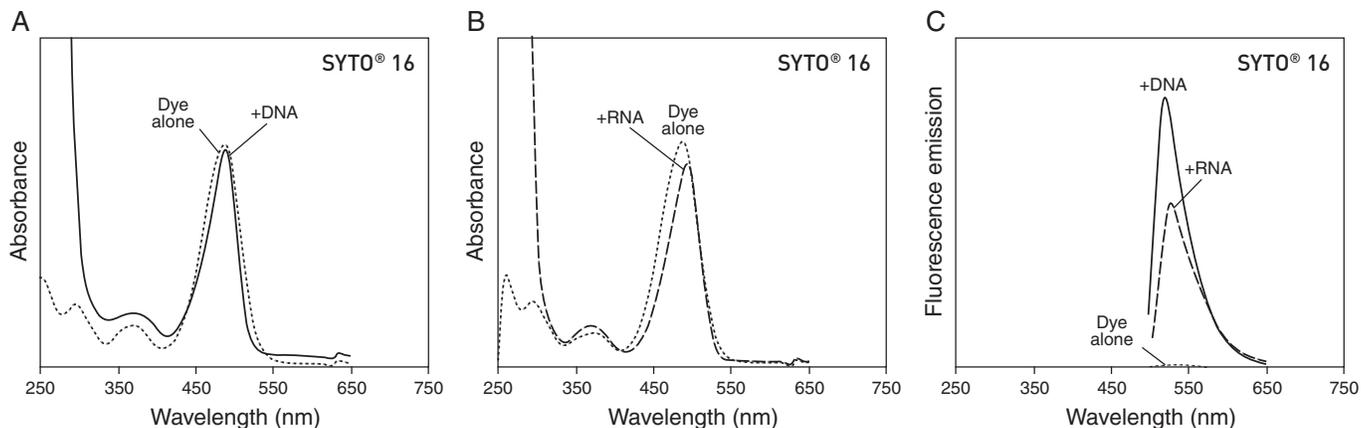
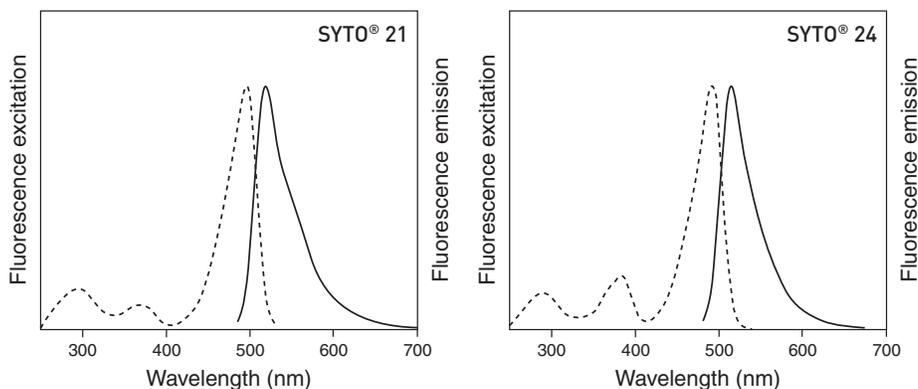


Figure 4 Spectra of SYTO® 21 and 24 Green-Fluorescent Nucleic Acid Stains

Normalized fluorescence excitation and emission spectra with dyes bound to DNA.



Product list Current prices may be obtained from our website or from our Customer Service Department.

Cat. no.	Product name	Unit size
S34854	SYTO® 9 green fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL
S7573	SYTO® 11 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S7574	SYTO® 12 green fluorescent nucleic acid stain *4 mM solution in DMSO*	315 µL
S7575	SYTO® 13 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S7576	SYTO® 14 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S7578	SYTO® 16 green fluorescent nucleic acid stain *1 mM solution in DMSO*	250 µL
S7556	SYTO® 21 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S7559	SYTO® 24 green fluorescent nucleic acid stain *5 mM solution in DMSO*	250 µL
S34855	SYTO® BC green fluorescent nucleic acid stain *5 mM solution in DMSO*	100 µL
S7572	SYTO® Green-Fluorescent Nucleic Acid Stain Sampler Kit *SYTO® dyes 11-14, 16, 21, 24 *50 µL each*	1 kit

Purchaser notification

Corporate headquarters

5791 Van Allen Way
Carlsbad, CA 92008
USA
Phone: +1 760 603 7200
Fax: +1 760 602 6500
Email: techsupport@lifetech.com

European headquarters

Inchinnan Business Park
3 Fountain Drive
Paisley PA4 9RF
UK
Phone: +44 141 814 6100
Toll-Free Phone: 0800 269 210
Toll-Free Tech: 0800 838 380
Fax: +44 141 814 6260
Tech Fax: +44 141 814 6117
Email: euroinfo@invitrogen.com
Email Tech: eurotech@invitrogen.com

Japanese headquarters

LOOP-X Bldg. 6F
3-9-15, Kaigan
Minato-ku, Tokyo 108-0022
Japan
Phone: +81 3 5730 6509
Fax: +81 3 5730 6519
Email: jpinfo@invitrogen.com

Additional international offices are listed at
www.lifetechnologies.com

These high-quality reagents and materials must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Read the Safety Data Sheet provided for each product; other regulatory considerations may apply.

Obtaining support

For the latest services and support information for all locations, go to www.lifetechnologies.com.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (techsupport@lifetech.com)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

SDS

Safety Data Sheets (SDSs) are available at www.lifetechnologies.com/sds.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.lifetechnologies.com/support and search for the Certificate of Analysis by product lot number, which is printed on the product packaging (tube, pouch, or box).

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Disclaimer

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Hoechst is a registered trademark of Hoechst GmbH. All other trademarks are the property of Thermo Fisher Scientific and its subsidiaries.

Life Technologies is a Thermo Fisher Scientific brand. © 2014 Thermo Fisher Scientific Inc. All rights reserved.