

RediPlate™ 96 Nucleic Acid Stain Sampler Microplate (R-32715)

Quick Facts

Storage upon receipt:

- ≤−20°C
- Protect from light
- Desiccate

Ex/Em: See Table 2

Introduction

Molecular Probes prepares an extensive assortment of nucleic acid-binding cyanine dyes, most of which have been developed in our own research laboratories. Among these are the SYTO® series of dyes, which are relatively low-affinity nucleic acid stains that passively diffuse through the membranes of most cells. These UV- or visible light–excitable 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.^{1–4} The SYTO dyes may also be useful for nucleic acid detection in solution, in electrophoretic gels, on blots, on microarrays and in many other applications.⁵ Because of their relatively low nucleic acid binding affinity, SYTO dyes stain a wider variety of cellular targets than dyes such as Hoechst 33342, YO-PRO®-1 and YOYO®-1, and cellular staining behavior of SYTO dyes can be variable and difficult to predict *a priori*. Consequently, extensive dye screening is beneficial in developing new applications for these dyes. The RediPlate™ 96 nucleic acid stain sampler microplate is designed to facilitate this process by providing 36 samples of different SYTO dyes pre-dispensed in a 96-well microplate. The plate also contains samples of SYBR® Green I, SYBR Green II and PicoGreen® dyes. Although these three dyes have primarily been developed for detection of nucleic acids in electrophoretic gels or in solution, they have also proven to be useful in cellular staining applications.^{6,7} Also included are samples of the amine-reactive SYBR 101 and SYBR 103 nucleic acid binding dyes. Finally, samples of six other nucleic acid binding dyes (Hoechst 33342, SYTOX® Green, SYTOX Orange, SYTOX Blue, propidium iodide and hexidium iodide) are provided for indicating cell viability and as references for the cellular staining behavior of the SYTO dyes. The general characteristics of the dyes provided in the RediPlate 96 nucleic acid stain sampler are summarized in Table 1.

Table 1. General characteristics of the dyes provided in the RediPlate 96 nucleic acid stain sampler.

Type	Number of dyes provided *	Characteristics
SYTO dyes	36	Membrane-permeant nucleic acid binding dyes, multiple fluorescent colors
SYBR Green I, SYBR Green II, and PicoGreen dyes	3	Dyes primarily used for nucleic acid detection on gels and for quantitation of DNA in solution
SYBR 101 and 103	2	Nucleic acid binding dyes with amine-reactive succinimidyl ester groups †
Hoechst 33342	1	Blue-fluorescent, membrane permeant nuclear stain
SYTOX Blue, SYTOX Green, SYTOX Orange, propidium iodide	4	Membrane-impermeant nucleic acid-binding dyes for detecting dead cells
Hexidium iodide	1	Membrane-permeant analog of propidium iodide

* Duplicate samples of 47 dyes are provided, plus two empty wells for fluorescence background measurements, making up the total of 96 wells.

† Amine reactivity of these dyes may diminish during prolonged storage of the microplate.

Materials

Contents

The RediPlate 96 nucleic acid stain sampler consists of one 96-well microplate containing duplicate samples of 47 different nucleic acid-binding dyes and two empty wells for fluorescence background measurements. The layout of the microplate is shown in Table 2 and is described in more detail below. The amount of dye in each well is calibrated to yield a concentration of approximately 20 μM after solubilization in 100 μL of a suitable solvent, typically dimethylsulfoxide (DMSO) or aqueous buffer.

Storage and Handling

Upon receipt, store the microplate at ≤−20°C, desiccated and protected from light in the foil pouch in which the product is shipped.

Microplate Characteristics

The layout of the RediPlate 96 nucleic acid stain sampler is shown in Table 2. Adjacent columns (e.g., columns 1 and 2) contain duplicate dye samples. The microplate is arranged such that dyes in a particular column have generally similar fluorescence excitation and emission wavelength characteristics. Each column of 8 wells is provided as a removable strip. Strips can be rearranged or removed from the plate according to user requirements.

Experimental Protocols

The RediPlate 96 nucleic acid stain sampler is intended primarily as a content source that can potentially be utilized in several ways. Two methods are described, below.

Protocol 1. The dyes may be dissolved in 100 μL of DMSO per well to yield approximately 20 μM stock solutions. Aliquots from these stock solutions can be transferred to microplate wells containing cultured cells. For example, the addition of a 1 μL volume of one stock solution in DMSO to 100 μL of culture medium will yield a staining solution of approximately 0.2 μM dye containing 1% DMSO.

Protocol 2. The dyes may be dissolved in 100 μL of aqueous buffer or medium (all the dye samples contain an inert excipient to facilitate aqueous solubilization). The resulting aqueous stock solutions (approximately 20 μM) may be transferred to another

microplate containing cultured adherent cells. Alternatively, portions of cells in suspension may be added to aqueous stock solutions in the dye source microplate. After incubation, fluorescence measurements can then be carried out directly in the source microplate. In this case, medium may be added to the empty wells A5 and A6 for measurements of background fluorescence.

Protocol 2 typically yields a much higher staining concentration than Protocol 1. For example, Protocol 2 yields a staining concentration of $\sim 10 \mu\text{M}$ from a 1:1 mixture of the aqueous dye solution and a cell suspension; whereas, Protocol 1 yields a staining concentration of $\sim 0.2 \mu\text{M}$ from 1 μL of the DMSO stock solution added to 100 μL of a cell suspension. The provision of duplicate dye samples allows both procedures to be tested if desired. Because the optimal dye concentration varies from dye to dye and from application to application, the set of dyes should be screened over a broad range of final concentrations, e.g., from 0.01 to 10 μM .

After a suitable incubation period (typically 5–60 minutes), fluorescence measurements may be performed using a standard microplate reader. Note that the dyes provided in the RediPlate 96 nucleic acid stain sampler exhibit varying degrees of fluorescence enhancement upon binding to nucleic acids and other cellular targets. Therefore, background fluorescence signals from aqueous dye staining solutions should normally be quite low and washing of stained cells will not be necessary in many cases. The fluorescence excitation and emission characteristics of the dyes vary quite widely. Suitable excitation and emission wavelength settings for individual dyes are listed in Table 2.

Table 2. Layout of the RediPlate 96 nucleic acid stain sampler microplate.

	Columns *					
	1 and 2	3 and 4	5 and 6	7 and 8	9 and 10	11 and 12
Row A	SYTOX Blue 445/470 S-11348	SYTOX Green 504/523 S-7020	Blank	Propidium iodide 530/625 P-1304	SYTOX Orange 547/570 S-11368	Hexidium iodide 518/600 H-7593
Row B	Hoechst 33342 350/461 H-1399	SYTO 9 483/503 S-34854	SYTO 10 484/505 S-32704	SYBR 103 486/526 S-21502	SYTO 86 528/556 S-32707	SYTO 64 598/620 S-11346
Row C	SYTO 45 452/484 S-11356	SYTO 16 488/518 S-7578	SYTO 25 521/556 S-7560	SYBR 101 494/519 S-21500	SYTO 85 567/583 S-11366	SYTO 63 654/675 S-11345
Row D	SYTO 44 445/472 S-11355	SYTO 15 516/546 S-7577	SYTO 24 490/515 S-7559	PicoGreen 503/523 P-7581	SYTO 84 567/582 S-11365	SYTO 62 649/680 S-11344
Row E	SYTO 43 427/464 S-11354	SYTO 14 517/549 S-7576	SYTO 23 499/520 S-7558	SYTO 26 497/534 S-32705	SYTO 83 543/559 S-11364	SYTO 61 620/647 S-11343
Row F	SYTO 42 430/460 S-11353	SYTO 13 488/509 S-7575	SYTO 22 515/535 S-7557	SYTO 27 495/537 S-32706	SYTO 82 541/560 S-11363	SYTO 60 652/678 S-11342
Row G	SYTO 41 426/455 S-11352	SYTO 12 500/522 S-7574	SYTO 21 494/517 S-7556	SYBR Green II 497/520 S-7568	SYTO 81 530/544 S-11362	SYTO 59 622/645 S-11341
Row H	SYTO 40 419/445 S-11351	SYTO 11 508/527 S-7573	SYTO 20 512/530 S-7555	SYBR Green I 498/522 S-7567	SYTO 80 531/545 S-11361	SYTO 17 621/634 S-7579

* Adjacent columns (e.g., columns 1 and 2) contain duplicate dye samples. Each cell in the figure corresponds to an adjacent pair of microplate wells. For each pair of wells, the following information is listed: Line 1, name of dye; Line 2, fluorescence excitation and emission maxima, in nm, for nucleic acid-bound dye; Line 3, Molecular Probes' catalog number for individual dye samples.

References

1. Cytometry 50, 249 (2002); 2. Methods 18, 222 (1999); 3. Lett Appl Microbiol 34, 182 (2002); 4. Cytometry 41, 223 (2000); 5. Nucleic Acids Res 29, e41 (2001); 6. Appl Environ Microbiol 66, 3790 (2000); 7. Aquat Microbial Ecol 14, 113 (1998).

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

Cat #	Product Name	Unit Size
R-32715	RediPlate™ 96 nucleic acid stain sampler *one 96-well microplate*	each

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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.

Please visit our Web site — www.probes.com — for the most up-to-date information.

Molecular Probes, Inc.

29851 Willow Creek Road, Eugene, OR 97402
Phone: (541) 465-8300 • Fax: (541) 335-0504

Customer Service: 6:00 am to 4:30 pm (Pacific Time)

Phone: (541) 335-0338 • Fax: (541) 335-0305 • order@probes.com

Toll-Free Ordering for USA and Canada:

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Technical Assistance: 8:00 am to 4:00 pm (Pacific Time)

Phone: (541) 335-0353 • Toll-Free (800) 438-2209
Fax: (541) 335-0238 • tech@probes.com

Molecular Probes Europe BV

Poortgebouw, Rijnsburgerweg 10
2333 AA Leiden, The Netherlands
Phone: +31-71-5233378 • Fax: +31-71-5233419

Customer Service: 9:00 to 16:30 (Central European Time)

Phone: +31-71-5236850 • Fax: +31-71-5233419
eurorder@probes.nl

Technical Assistance: 9:00 to 16:30 (Central European Time)

Phone: +31-71-5233431 • Fax: +31-71-5241883
eurotech@probes.nl

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