Dimeric Cyanine Nucleic Acid Stains

Quick Facts

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

Unit Size: 200 µL of a 1 mM solution in DMSO

Introduction

Molecular Probes' dimeric cyanine nucleic acid dyes (Table 1) are among the highest sensitivity fluorescent probes available for nucleic acid staining. In addition to their high affinity for nucleic acids, TOTO®-1 dye and the other cyanine dimers are essentially nonfluorescent in the absence of nucleic acids and exhibit 100- to 1000-fold fluorescence enhancements upon binding to DNA.[1,2] The extinction coefficients and fluorescence quantum yields of the cyanine dimers bound to DNA are high, resulting in very bright fluorescence signals.[1]

The dimeric cyanine dyes are useful for many applications. Their bright fluorescence signals and low backgrounds make them ideal for staining nucleic acids on solid supports, such as microarrays. They are also useful as nuclear and chromosome counterstains for multicolor fluorescence labeling experiments. The extraordinary stability of the dye–nucleic acid complexes [1,3] makes it possible to prestain nucleic acid samples prior to gel [4–8] or capillary electrophoresis.[9–13] Protocols for some of these techniques are outlined in *Experimental Protocols* (below). Other applications are described in our *Handbook of Fluorescent Probes and Research Chemicals*, found at our Web site (www.probes.com).

Materials

Contents

The dimeric cyanine nucleic acid stains are supplied in a unit size of 200 µL as 1 mM solutions in dimethylsulfoxide (DMSO); except for POPO™-3 dye, which is supplied as a 1 mM solution in dimethylformamide (DMF). The Nucleic Acid Stains Dimer Sampler Kit (N-7565) contains 10 µL of a 1 mM solution of each dye, with the exception of the LOLO™-1 and JOJO™-1 dyes.

Storage and Handling

Upon receipt, the dye solution should be stored at -20°C in a desiccator. Stored properly, the expected shelf life of these stock solutions is 6–12 months. Allow all solutions to warm to room temperature and mix thoroughly before use. Long-term storage of working solutions in 100% aqueous media should be avoided. These cationic dyes appear to be readily adsorbed out of aqueous solutions onto surfaces (particularly glass) but are very stable once complexed to nucleic acids.

Spectral Characteristics

The fluorescence spectra of the ten dimeric cyanine nucleic acid stains cover the entire visible wavelength range, as summarized in Table 1. The table also lists laser-line sources suitable for excitation and detection of these dyes.

Table 1. *Spectral characteristics of cyanine dimer nucleic acid stains bound to dsDNA.*

Name	Catalog #	MW *	Abs † (nm)	ϵ_{max} ‡ ($cm^{-1}M^{-1}$)	Em † (nm)	QY §	Excitation Light Source (nm)
POPO-1	P-3580	1171	434	92,400	456	0.60	Hg arc (only) 436
BOBO-1	B-3582	1203	462	113,600	481	0.22	He-Cd 442
YOYO-1	Y-3601	1271	491	98,900	509	0.52	Ar 488
TOTO-1	T-3600	1303	514	117,000	533	0.34	Ar 514
JOJO-1	J-11372	1273	529	171,400	545	0.44	Nd: YAG 532
POPO-3	P-3584	1223	534	146,400	570	0.46	He-Ne 543
LOLO-1	L-11376	1463	565	108,400	579	0.40	Kr 568
BOBO-3	B-3586	1255	570	147,800	602	0.39	Kr 568
YOYO-3	Y-3606	1323	612	167,000	631	0.15	He-Ne 594
TOTO-3	T-3604	1355	642	154,100	660	0.06	He-Ne 633

***** Molecular weight. **†** Absorption (Abs) and fluorescence emission (Em) maxima. Full absorbance and emission spectra can be found at our Web site (www.probes.com). **‡** Molar extinction coefficient. **§** Fluorescence quantum yield determined relative to fluorescein in 0.1 M NaOH (QY=0.92). Abs, Em, ϵ_{max} and QY determined for DNA complexes in 10 mM Tris, 1 mM EDTA, 50 mM NaCl, pH 7.4.

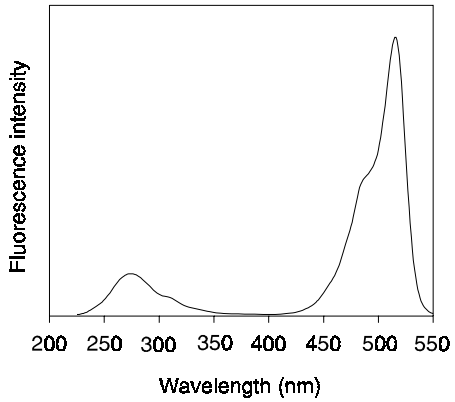

Figure 1. *Fluorescence excitation spectrum of TOTO-1 complexed with DNA at a ratio of 1 dye:50 base pairs.*

POPO-1, BOBO™-1, YOYO®-1 and TOTO-1 dyes may also be used with ultraviolet excitation sources. As shown in Figure 1, the fluorescence excitation spectrum of DNA-bound TOTO-1 dye has a short-wavelength peak at about 275 nm. POPO-1, BOBO-1 and YOYO-1 dyes exhibit similar ultraviolet excitation peaks. However, the longer-wavelength dyes (POPO-3, BOBO-3, YOYO-3, TOTO-3, JOJO-1 and LOLO-1 dyes) are only weakly excited by ultraviolet sources.

Experimental Protocols

Staining DNA Microarrays for Quality Control

Techniques for creating arrays of nucleic acids on solid supports vary widely in reproducibility. This variability in the spotting can lead to artifactual changes in signal. Fluorescent nucleic acid stains provide a simple and direct method for analyzing spotted DNA on solid supports (Figure 2). The following procedure is adapted from a published experiment using SYBR Green I nucleic acid gel stain [14] (S-7563, S-7567), but should be generally applicable for use with any of the dimeric cyanine dyes.

1.1 Dilute the stain 10,000-fold in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0) or TBE buffer (45 mM Tris-borate, 1 mM EDTA, pH 8.0).

1.2 Cover the microarray with diluted stain and incubate at room temperature for 2–3 minutes.

1.3 Wash the microarray 3–4 times with TE or TBE buffer.

1.4 Spin-dry the microarray for 1–2 minutes.

1.5 Scan the microarray using filter sets appropriate for the dye (see Table 1).

1.6 To wash the stain off of the slide, incubate the slide at room temperature for 1 hour in a solution of 0.1% SDS, 10 mM Tris, 1 mM EDTA, pH 7.5. After drying, the slides can be used for hybridization.

Counterstaining Chromosomes with YOYO-1 dye

The following protocols were optimized for use with YOYO-1 dye; however, these protocols, or variations of these protocols, could potentially be used with other dimeric cyanine dyes.

Before counterstaining, prepare and hybridize chromosome spreads on microscope slides according to standard procedures and rinse the spreads briefly in water.

Counterstaining in *SlowFade*® or DABCO-containing mounting medium.

2.1 Dilute the YOYO-1 dye to 2.4 nM in phosphate-buffered saline (PBS) and apply 200 µL of this diluted YOYO-1 solution directly to the rinsed slide.

2.2 Incubate the sample with the stain at room temperature for at least 10–20 minutes for optimal staining.

2.3 Rinse the slide briefly with water to eliminate unbound dye and PBS. Remove excess liquid from the slide.

2.4 Apply two drops of *SlowFade* reagent to the slide, one near the top and one near the bottom, and allow the antifade reagent to evenly disperse over the surface of the slide. Place a 24 × 50 mm coverslip on the slide and seal it with nail polish or wax. For an improved signal-to-background ratio, allow the sample to incubate in the *SlowFade* solution for 30 minutes prior to visualization.

2.5 View the sample with a fluorescence microscope equipped with a standard fluorescein filter set.

2.6 Slides can be stored protected from light at room temperature or at 4°C.

Counterstaining in *p*-phenylenediamine (PPD)-containing mounting medium.

3.1 Allow rinsed slides to air dry.

3.2 Serially dilute the YOYO-1 dye to a final concentration of 0.1 µM in PPD-containing mounting medium. A simple PPD-containing medium contains 2 mg/mL PPD in a solution of 50% PBS and 50% glycerol; this mounting medium oxidizes rapidly and should be stored (only for short periods of time) frozen at -20°C. The YOYO-1 dye/mounting medium solution should be used immediately.

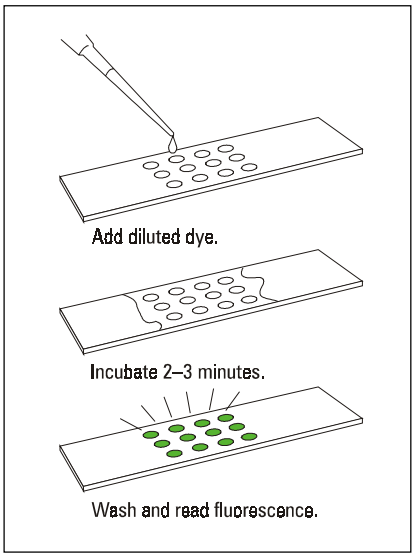

Figure 2. *Procedure for staining microarray slides with nucleic acid dyes.*

3.3 Apply 20–30 µL of the diluted YOYO-1 dye/mounting medium solution directly to the dry slide. Place a 24 × 50 mm coverslip on the slide and seal the coverslip with nail polish or wax.

3.4 View the sample with a fluorescence microscope equipped with a standard fluorescein filter set.

3.5 When the slide is stored at 4°C, counterstaining becomes stronger. To avoid this signal enhancement, remove the coverslip once the slide has been examined and wash the slide briefly with methanol or ethanol to rinse away the mounting medium. Air dry the slide and store it at 4°C, protected from light. The sample can be examined again after re-embedding it in the PPD-containing medium.

Prestaining DNA before Analysis by Gel Electrophoresis

4.1 Add DNA to a solution of the dye in 40 mM Tris-acetate, 2 mM EDTA, pH 8.0 to yield a DNA concentration of 0.05–1.5 ng/µL and a **minimum** ratio of 5:1, DNA base pairs (bp) to dye molecules. A 5:1 ratio corresponds to addition of 1 ng of DNA to 3 µL of 0.1 µM dye (prepared by 1:10,000 dilution of the 1 mM stock solution).

- The mixing sequence (add DNA to dye) has a significant impact on the eventual electrophoretic resolution.[1,8]

- Prepare aqueous solutions by dilution from the DMSO stock solution immediately before they are required for use. Aqueous solutions of dimeric cyanine dyes without added DMSO are unstable due to surface adsorption. Prepare aqueous solutions in plastic rather than glass containers.

- Dye solutions should be reasonably stable for up to 1 hour at room temperature.

- Once the DNA/dye complexes are formed, they are stable under standard laboratory electrophoresis conditions. The DNA/dye complex in solution is stable indefinately when stored at 4°C.

- DNA:dye ratios of less than 5 bp to 1 dye molecule may result in precipitation of the complexes, severe streaking and band-splitting during electrophoresis, and do not result in greater detection sensitivity.

4.2 Incubate the samples for 60 minutes at room temperature.

4.3 Immediately before electrophoresis, add one volume of 15% (wt/vol) Ficoll in water to three volumes of sample. Avoid addition of tracking dyes that have significant fluorescence (e.g., bromophenol blue, xylene cyanole) to the same lanes as cyanine dye–labeled DNA samples.

4.4 Load aliquots onto gels.

4.5 Perform electrophoresis in the dark, using the buffer described in step 1.1.

4.6 Visualize the gels by using UV trans- or epi-illumination or a laser-excited fluorescence gel scanner.[4,8,9]

References

1. Nucleic Acids Res 20, 2803 (1992); **2.** J Phys Chem 99, 17936 (1995); **3.** Proc Natl Acad Sci USA, 87, 3851 (1990); **4.** Nucleic Acids Res 21, 5720 (1993); **5.** Nature 359, 859 (1992); **6.** Science 257, 886 (1992); **7.** Methods Enzymol 217, 414 (1993); **8.** BioTechniques 10, 616 (1991); **9.** J Chromatogr B 732, 365 (1999); **10.** Anal Chem 66, 1424 (1994); **11.** Anal Biochem 215, 163 (1993); **12.** Anal Biochem 224, 140 (1995); **13.** J Chromatogr B 658, 271 (1994); **14.** BioTechniques 29, 78 (2000).

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

Cat #	Product Name	Unit Size
B-3582	BOBO™-1 iodide (462/481) *1 mM solution in DMSO*	200 µL
B-3586	BOBO™-3 iodide (570/602) *1 mM solution in DMSO*	200 µL
J-11372	JOJO™-1 iodide (529/545) *1 mM solution in DMSO*	200 µL
L-11376	LOLO™-1 iodide (565/579) *1 mM solution in DMSO*	200 µL
N-7565	Nucleic Acid Stains Dimer Sampler Kit	1 kit
P-3580	POPO™-1 iodide (434/456) *1 mM solution in DMSO*	200 µL
P-3584	POPO™-3 iodide (534/570) *1 mM solution in DMF*	200 µL
S-2828	*SlowFade*® Antifade Kit	1 kit
S-7461	*Slow Fade*® *Light* Antifade Kit	1 kit
T-3600	TOTO®-1 iodide (514/533) *1 mM solution in DMSO*	200 µL
T-3604	TOTO®-3 iodide (642/660) *1 mM solution in DMSO*	200 µL
Y-3601	YOYO®-1 iodide (491/509) *1 mM solution in DMSO*	200 µL
Y-3606	YOYO®-3 iodide (612/631) *1 mM solution in DMSO*	200 µL

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