Reference Dye Sampler Kit (R-14782)

Quick Facts

Storage upon receipt:

- Room temperature
- Protect from light

Introduction

Fluorescence intensities are influenced by a variety of sample-related and instrument-related parameters. Consequently, comparisons between data acquired on different instruments or with long intervals (several days or more) between measurements must be made with reference to standard fluorescent materials.[1-3] Molecular Probes' Reference Dye Sampler Kit provides samples of five extensively characterized fluorescence standards (Table 1) with emission spectra covering the entire visible wavelength range (Figure 1). Reference absorption and fluorescence emission spectra for all five compounds, measured in our laboratories, are available for downloading at our Web site (see *Applications*).

Materials

All five fluorescent standards are supplied as spectrophotometrically determined 1 mM stock solutions in 1 mL units. The compositions of these solutions are as follows:

- **Quinine sulfate** (Component A), in 0.1 M sulfuric acid (H_2SO_4)
- **Fluorescein** (Component B), in dimethylsulfoxide (DMSO)
- **5-Carboxytetramethylrhodamine** (Component C), in DMSO
- **Sulforhodamine 101** (Component D), in DMSO
- **Nile blue perchlorate** (Component E), in DMSO

All five stock solutions may be stored at room temperature, protected from light.

Properties

The following notes describe the impact of various environmental factors on the fluorescence quantum yields (QY) of the standards supplied in the Reference Dye Sampler Kit. Measured fluorescence intensities of standard solutions, which are directly related to the QY, are affected in the same way.

Quinine Sulfate. The QY of quinine sulfate depends somewhat on the concentration of dilute acid in which it is dissolved.[1-2] For example, QY in 0.1 M perchloric acid is 0.60 compared to 0.55 in 0.5 M sulfuric acid. Quinine sulfate's QY is markedly temperature-dependent (-0.25% per °C) but is only minimally susceptible to quenching by dissolved oxygen.[4]

Fluorescein. The QY of fluorescein is strongly pH-dependent at pH <8. Fluorescence is maximal in alkaline solutions with pH >9 (Table 1). Quenching by dissolved oxygen is minimal.[4]

5-Carboxytetramethylrhodamine (5-CTMR) and nile blue. Fluorescence quantum yields of dyes with unhindered dialkylamine substituents such as 5-CTMR and nile blue are strongly temperature-dependent (the QY decreases as temperature increases).

Sulforhodamine 101. Sulforhodamine 101 has rigidized amine substituents; consequently its QY is almost completely independent of temperature.[5]

Table 1. *Spectroscopic data for Reference Dye Sampler Kit components.*

Component	Solvent [1]	Abs (nm) [2]	Em (nm) [2]	QY [3]	Reference
Quinine sulfate, dihydrate	0.5 M H_2SO_4	349	460	0.55	J Phys Chem 75, 991 (1971)
Fluorescein	0.1 M NaOH	493	513	0.92	Trans Faraday Soc 53, 646 (1957)
5-CTMR [4]	methanol	543	567	0.68	J Phys Chem 83, 696 (1979)
Sulforhodamine 101	ethanol	578	593	1.00	J Phys Chem 84, 1871 (1980)
Nile blue perchlorate	acidic ethanol [5]	631	660	0.27	J Luminescence 24, 709 (1981)

1. Solvent used for measurements of spectroscopic parameters reported in columns 3–5. Note that these solvents are different from those used to prepare the stock solutions supplied in the kit (see section 2). **2. Abs**orption and fluorescence **Em**ission maxima. **3.** Fluorescence quantum yield at 22°C in the solvent specified in column 2. Values are provided for reference purposes only. We do not certify that the QY values of the materials supplied in this kit are identical to those listed. **4.** CTMR = carboxytetramethylrhodamine. **5.** 0.5% (v/v) 0.1 M HCl in ethanol.

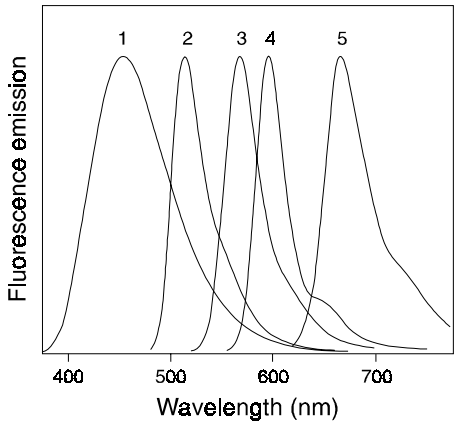

Figure 1. *Normalized fluorescence emission spectra of the five components of the Reference Dye Sampler Kit.* ***1.*** *Quinine sulfate,* ***2.*** *fluorescein,* ***3.*** *5-carboxytetramethylrhodamine,* ***4.*** *sulforhodamine 101,* ***5.*** *nile blue.*

Applications

Fluorescence Microplate Readers

To prepare working samples for fluorescence microplate readers, dilute a small amount of the stock solution supplied at least 100-fold into the measurement solvent indicated in the second column of Table 1 (note **A**). For example, add 10 µL of the stock solution to 2 mL of measurement solvent to prepare a 5 µM working sample (note **B**). This will provide sufficient sample for 10–20 microplate wells (100–200 µL/well). Serial dilutions of a 5 µM working sample can be used to check fluorescence intensity–dye concentration linearity (note **C**). Suitable excitation and emission filters for measurements can be selected using the peak wavelengths listed in Table 1 as a guide.

Spectrofluorometers

To prepare working samples for spectrofluorometers, dilute a small amount of the stock solution supplied at least 100-fold into the measurement solvent indicated in the second column of Table 1 (note **A**). For example, add 10 µL of stock solution to 10 mL of measurement solvent to prepare a 1 µM working sample (note **B**). Typically, 2 mL of working sample is required for a single measurement in conventional 1 cm^2 cuvettes. Serial dilutions of a 1 µM working sample can be used to check fluorescence intensity–dye concentration linearity (note **C**). Suitable excitation and emission wavelength ranges for measurements can be selected using the peak wavelengths listed in Table 1 as a guide.

Fluorescence Quantum Yield (QY) Measurements

The most readily implemented methods for determination of QY involve comparing the spectrally integrated fluorescence emission of an experimental sample with that of a standard compound. Detailed descriptions of these so-called secondary methods and assessments of the inherent experimental errors can be found in the literature.[1,4] All the compounds supplied in the Reference Dye Sampler Kit have documented utility as QY standards (Table 1). However it should be noted that we do not certify that the compounds supplied in this kit have the exact QY values listed in Table 1 (note **D**).

Downloading Reference Spectra from our Web Site

1.1 Access Molecular Probes' Web site (www.probes.com).

1.2 Select the **Search** option.

1.3 Click the **catalog numbers** option in the **Product Search** area and enter the catalog number 14782 in the dialog box.

1.4 Click **Search Products**. A list of links to product information pages will be displayed, among which will be links to the spectra of each of the five reference dye components of this kit.

1.5 Click the spectra link for the reference dye of interest. A plot of the absorption and fluorescence emission spectra (.gif format) is displayed.

1.6 The page on which the spectral plot is displayed also contains a link to an ASCII text file containing the digital (x,y) data used to generate the plot (note **E**). This file can be downloaded and imported into a spreadsheet program.

Notes

[A] Use of other measurement solvents (e.g. substitution of aqueous solvents for alcohols or *vice versa*) may produce significant changes in spectral maxima or QY. Some specific examples are described in *Properties*.

[B] If greater precision is desired, use a 2-stage dilution. For example to dilute 1 mM to 1 µM, add 50 µL of stock solution to 1 mL of measurement solvent, then add 100 µL of this first stage dilution to a further 4.7 mL of measurement solvent.

[C] For fluorescence microplate readers, a linear measurement range from 5×10^{-6} M (5 µM) – 1×10^{-12} M is typical. For spectrofluorometers, a typical linear measurement range is 1×10^{-6} M (1 µM) – 1×10^{-13} M. Actual results will vary depending on instrument design and dye characteristics.

[D] Quinine sulfate, dihydrate with a certified fluorescence emission spectrum and QY is available from the United States National Bureau of Standards (SRM 936).[6]

[E] The downloadable ASCII text files contain 4 data columns headed wl (wavelength in nm), abs (absorbance), wl (wavelength in nm) and em (fluorescence emission intensity). The y-values (absorbance and fluorescence emission intensity) are normalized to an arbitrary peak value of 100. Spectral data contained in these files was obtained in our laboratories using a Perkin-Elmer Lambda 14 spectrophotometer (absorption spectra) and a Hitachi F-4500 spectrofluorometer (fluorescence emission spectra). Fluorescence emission spectra were corrected for wavelength-dependent variations in detection sensitivity using a correction curve supplied by the instrument manufacturer.

References

1. *Standards in Fluorescence Spectrometry*, J.N. Miller, Ed., Pub. Chapman and Hall (1981); **2.** Pure Appl Chem 60, 1107 (1988); **3.** *Luminescence Applications in Biological, Chemical, Environmental and Hydrological Sciences (ACS Symposium Series 383*), M.C. Goldberg, Ed., American Chemical Society (1989), pp 98–126; **4.** J Phys Chem 75, 991 (1971); **5.** J Phys Chem 84, 1871 (1980); **6.** NBS Special Publication 260-64 (1980).

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

Cat #	Product Name	Unit Size
R-14782	Reference Dye Sampler Kit *five 1 mM solutions, 1 mL each*	1 kit

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