FluoSpheres® Fluorescent Microspheres for Tracer Studies

Quick Facts

Storage upon receipt:

- 4°C
- Do not freeze
- Protect from light

Abs/Em: See Table 1

Introduction

FluoSpheres® fluorescent microspheres for tracer studies are 1.0 µm polystyrene beads maximally loaded with fluorescent dyes. Because the dye content of these microspheres is much higher than that of other fluorescent microspheres, stronger signals can be generated using fewer microspheres per tracing experiment. Recently, aerosols containing these heavily dye-loaded fluorescent microspheres have been used to acquire high-resolution maps of regional pulmonary ventilation.[1,2] Transport of fluorescent microspheres through tissues can be quantified using methods that have been developed for regional blood flow determination. The microspheres and the fluorescent dyes they contain are extracted from the tissue. Fluorescence is then quantified using readily available instrumentation such as spectrofluorometers or fluorescence microplate readers.

Materials

These products are uniform polystyrene microspheres with a nominal diameter of 1 µm; the actual measured diameter for each lot is printed on the product label. The microspheres are supplied in unit sizes of 5 mL, as suspensions at a concentration of 1.0×10^{10} microspheres per mL in distilled water with 0.02% thimerosal. Each microsphere reagent contains a single fluorescent dye whose fluorescence is well resolved from all of the others (Figure 1, Table 1). The exact excitation and emission spectra may vary somewhat with the solvent used to extract the fluorescent dyes (Table 1). Because of the heavy dye-loading, fluorescence in the beads is partially quenched. Maximum fluorescence occurs once the dyes have been released by dissolution of the beads.

Storage and Handling

The microspheres should be stored at 4°C, protected from light, when not in use. DO NOT FREEZE. Thimerosal has been added to maintain sterility; however, the use of sterile technique in withdrawing samples is required to avoid potential contamination. The microspheres are stable for at least one year, provided the recommended storage conditions are strictly observed.

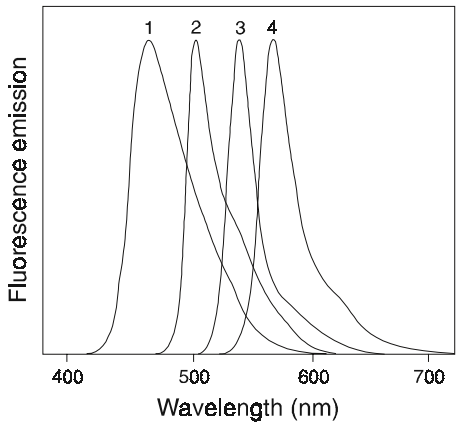

Figure 1. *Normalized fluorescence emission spectra of the dyes contained in the FluoSpheres® fluorescent microspheres for tracer studies, after extraction into 2-ethoxyethyl acetate (Cellosolve® acetate). The four types of microspheres represented are: 1) blue-green, 2) yellow-green, 3) orange and 4) red.*

Experimental Protocols

Overview

FluoSpheres beads are administered by appropriate techniques into the subject animal. The experimental conditions are planned and carried out in the same manner as if radioactive microspheres were used. After tissue samples are collected,

Table 1. *Absorption maximum and emission maximum wavelengths of FluoSpheres fluorescent microspheres for tracer studies.*

Cat #	Fluorescent Color *	Abs (nm) †	Em (nm) ‡
F-13080	Blue-green (430/465)	427	468
F-13081	Yellow-green (505/515)	495	505
F-13082	Orange (540/560)	534	554
F-13083	Red (580/605)	570	598

***** The number pairs next to the color in the product names are the nominal excitation and emission maxima, in nm, for the fluorescent beads suspended in water. † The absorption maxima were determined for dyes extracted from beads in Cellosolve acetate. Wavelengths may differ somewhat in other solvents. Excitation maxima are essentially the same as the absorption maxima; however, it is sometimes advisable to excite with slightly lower wavelengths, to obtain optimal peak resolution and to isolate emission from scattered excitation light. ‡ The emission maxima were determined for dyes extracted from beads in Cellosolve acetate.

the fluorescent microspheres can be directly extracted from lung tissue samples by use of an appropriate solvent. The fluorescence of the dye-containing solvent is then quantified with a fluorometer.

Materials That May Be Required But Are Not Provided

- 2-Ethoxyethyl acetate (Cellosolve® acetate) or xylene

Assay Procedure

Introducing Microspheres Into Tissue

Accurate measurements require that the microspheres are uniformly dispersed prior to use. Aggregation of the microspheres should be minimized by ultrasonicating the microsphere suspension before use and vigorously vortexing them prior to introduction into tissue. Microsphere suspensions may be diluted using distilled water. Solutions containing salts should not be used since they will cause the microspheres to aggregate. A protocol is described in the literature for introducing microspheres into lung tissues using aerosols.[1]

Dissolution of Fluorescent Microspheres

Since air-dried lung is less dense than other tissues, the fluorescent dyes can be extracted without first digesting the tissue. By soaking the tissue in an appropriate solvent, the polystyrene microspheres will dissolve, releasing the fluorescent dye. Appropriate solvents include 2-ethoxyethyl acetate (Cellosolve acetate) or xylene (note **A**). The tissue should be completely immersed and allowed to soak for at least 24 hours in an airtight vial to ensure against evaporation of the solvent and protected from light. Because the relative number of microspheres in each sample is determined from the concentration of extracted dye, it is critical that solvent volumes are kept constant from sample to sample.

Measurement and Quantitation of Fluorescence

1. Instrumentation: Almost any fluorometer that is equipped with a monochromator capable of scanning excitation and emission wavelengths from about 350 nm to 650 nm can be used to measure the fluorescence of the dissolved microspheres. Instruments of this type allow a wide selection of excitation and emission wavelengths with narrow bandpasses, resulting in good spectral separation of complex mixtures of dyes. Fluorometers with fixed-wavelength filter sets are not well suited, unless the filters have narrow bandpass (about 4 nm) and correspond to the excitation and emission properties of the FluoSpheres beads, which are summarized in Table 1. The samples can be measured in 1 cm pathlength cells, flow cells or, in some cases, 96-well microplates.

2. Measurement of Fluorescence: The fluorescence of the samples can be measured in two ways: 1) by exciting the dyes at specific wavelengths and recording the emission light at their respective maximum emission wavelengths, or 2) by scanning both the excitation and emission wavelengths simultaneously with a fixed wavelength interval between the excitation and emission (synchronous scan). Both methods have been shown to give excellent quantitation of the fluorescence of sample mixtures containing all of the fluorescent dyes used in FluoSpheres tracer reagents. Because each of the dyes has excitation and emission at distinct and well separated wavelengths from all of the others, it is necessary to measure only the peak emission intensity of each dye in order to obtain accurate quantitation. No special software is necessary to compensate for spectral spillover, because there is essentially none.[2] It is important to understand that measured fluorescence intensities and spectra will vary with each individual instrument due to the wavelength-dependent efficiency of both the excitation source lamp and the photomultiplier (detector).

Fluorescence Intensity: In dilute solutions, the intensity of the fluorescence signal is linearly proportional to the concentration of fluorescent dye (note **B**). The fluorescence signal from each tissue sample will be proportional to the number of microspheres dissolved in solution if the volume of solvent used for all tissue is constant. It is therefore critical that precise volumes of solvent are used for all samples (see also *Measurement and Quantitation of Fluorescence*).

Stokes Shift: The Stokes shift is the difference between the wavelengths for maximum excitation and for maximum emission. The dyes used in FluoSpheres beads have Stokes shifts ranging from about 10 nm to about 60 nm. The actual excitation wavelengths used in an experiment can be optimized empirically to give maximum fluorescence intensity, while retaining acceptable peak resolution (note **C**); often, these optimal excitation wavelengths are slightly lower than the absorption maxima given in Table 1.

Slit Widths: The slit width or bandpass regulates the amount of exciting light admitted to the sample and the amount of emission light that is recorded by the detector. An excitation wavelength of 530 nm with a slit width of 4 nm means that light with wavelength from 528–532 nm is used to excite the sample. Increasing the slits will increase the fluorescence signal intensity at a given wavelength, but can also result in interference (spectral spillover) from other dyes present in the solution.

3. Resolving Multiple Fluorescent Colors: The number of fluorescent colors that need to be resolved depends on the type of experiment carried out. It should be noted that detectors in fluorometers are often more sensitive to one part of the spectral range than another, and therefore the type of detector may dictate to some extent the choice of colors used in an experiment.

- **Synchronous Scanning:** The sample is placed in the instrument and analyzed in synchronous scan mode, using a wavelength offset of about 15 nm (note **D**). This method increases the resolution of closely spaced spectral peaks.[3] The relative number of microspheres is obtained from the maximum intensity of each peak.

- **Specific Excitation/Emission Pairs:** In this method, the sample is analyzed by exciting sequentially at the wavelength optimal for each dye component, and the peak emissions are recorded to give the relative number of microspheres (Table 1). This method may be very fast and simple in some instruments in which these parameters can be easily controlled by the software. This method may also give better overall signal intensities than the synchronous scan method.

Notes

[**A**] The solvents listed in section 3.2 are inexpensive and have been shown to readily dissolve the microspheres and the dyes. Do not confuse Cellosolve acetate (2-ethoxyethyl acetate) with ethyl Cellosolve (2-ethoxyethanol), which will not dissolve the microspheres. It has been reported by Dr. R. Glenny (FMRC) that 2-(2-ethoxy-ethoxy)ethyl acetate (Carbitol® acetate) works well for extracting all of the dyes used in FluoSpheres polystyrene microspheres for fluorescent tracing. Extraction of lung tissue directly with Carbitol acetate required approximately 96 hours before dye signals were stable, as compared to 48 hours for extraction with Cellosolve acetate. All potential solvents have not yet been tested, and therefore others may give satisfactory results; however, it is advantageous to use a solvent with low volatility to minimize evaporation. Dimethylsulfoxide and dimethylformamide are not suitable solvents. The microspheres will not dissolve in dimethylsulfoxide and the fluorescent dyes are not stable in dimethylformamide.

[**B**] If fluorescent dye solutions are too concentrated, quenching (reduction) of the fluorescent signal can occur as a result of physical interaction of the dye molecules in solution, leading to erroneous results. Usually, the solutions will be dilute enough that this will not occur, but if quenching is suspected, it can be confirmed by carrying out a twofold dilution of the sample in the extraction solvent. The fluorescence reading should be 50% of the original solution if quenching is not present. If quenching is present, the diluted solution will have greater than 50% of the fluorescence of the original solution.

[**C**] At equal concentrations, some of the fluorescent microspheres give a greater signal intensity than others when the recommended wavelength pairs from Table 1 are used. If it is desired to decrease one or more of the individual peak emission intensities so that all peaks are at about the same intensity, this can be done selectively by either reducing the microsphere concentration, moving the excitation to a shorter, less optimum wavelength or changing the slit width to a narrower setting. Optimal conditions can be worked out in control experiments using fixed numbers of pure beads. Likewise, correction factors can be determined to allow for the different signal intensities.

[**D**] Experiments have shown that a wavelength offset of about 15 nm between excitation and emission gives the greatest signal and separation of all of the colors in the solvent systems tested; however, if not all of the colors are used in the experiment, it may be possible to optimize this interval further.

Fluorescent Microsphere Resource Center

A Fluorescent Microsphere Resource Center (FMRC) has been established at the University of Washington (Seattle, WA) to compile and disseminate information on fluorescent microsphere techniques for measuring regional blood flow. The FMRC is a useful source for information on extracting the fluorescent dyes from FluoSpheres microspheres and performing fluorescence measurements. The FMRC center has developed a technical manual describing fluorescent microsphere methodology, and can be contacted by phone at (206) 685-9479, by fax at (206) 685-9480 or visit their Web site at http://fmrc.pulmcc.washington.edu/FMRC/fmrc.html.

References

1. J Appl Physiol 85, 2344 (1998); **2.** J Appl Physiol 88, 1551 (2000).

Product List

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

Cat #	Product Name	Unit Size
F-13080	FluoSpheres® polystyrene microspheres, 1.0 µm, blue-green fluorescent (430/465) *for tracer studies* *1.0x10^{10} beads/mL*	5 mL
F-13082	FluoSpheres® polystyrene microspheres, 1.0 µm, orange fluorescent (540/560) *for tracer studies* *1.0x10^{10} beads/mL*	5 mL
F-13083	FluoSpheres® polystyrene microspheres, 1.0 µm, red fluorescent (580/605) *for tracer studies* *1.0x10^{10} beads/mL*	5 mL
F-13081	FluoSpheres® polystyrene microspheres, 1.0 µm, yellow-green fluorescent (505/515) *for tracer studies* *1.0x10^{10} beads/mL*	5 mL

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