Revised: 13–March–2001

FluoSpheres® Fluorescent Microspheres

Quick Facts

Storage upon receipt:
- 4°C
- Do not freeze
- Protect from light

Ex/Em: See Table 2

Introduction

Molecular Probes' intensely fluorescent FluoSpheres® beads are manufactured using high-quality, ultraclean polystyrene microspheres. These microspheres are loaded with a variety of our proprietary dyes, making them the brightest fluorescent microspheres available. The FluoSpheres product line includes microspheres in eleven fluorescent colors, ranging from our UV-excitable blue to our He-Ne laser–excitable crimson and laser diode–excitable far red and infrared beads. We offer microspheres in a range of uniform sizes with negatively charged sulfate groups or with positively charged amine groups. Our carboxylate-modified microspheres are coated with a hydrophilic polymer containing multiple carboxylic acids for covalent attachment of ligands.

Table 1 provides a summary of Molecular Probes' fluorescent microspheres. The table conveniently presents catalog numbers, unit sizes and suspension densities for the various fluorescent colors, bead diameters and surface modifications of microspheres that we offer. We also offer biotin-, streptavidin-, NeutrAvidin™- and protein A–labeled FluoSpheres beads, as well as beads that are specifically designed for cell tracing and regional blood flow measurements.

Colors, Sizes and Surface Chemistries

Spectral Properties

Molecular Probes' FluoSpheres fluorescent microspheres contain dyes with excitation and emission wavelengths that cover the entire spectrum from the near ultraviolet to the near infrared. Figure 1 shows the normalized emission spectra for ten of our eleven fluorescent colors of FluoSpheres beads. The approximate excitation and emission maxima of the microspheres are indicated in Table 1. Highlights from the FluoSpheres product line include:

- Our new **blue fluorescent FluoSpheres beads** with excitation/emission maxima of 350/440 nm contain an improved blue fluorescent dye that provides superior brightness and a longer shelf life. We also offer blue fluorescent FluoSpheres beads with slightly shorter-wavelength fluorescence spectra (excitation/emission maxima = 365/415 nm).
- Our **yellow-green fluorescent FluoSpheres beads** are excited very efficiently using the 488 nm spectral line of the argon-ion laser and have exceptionally intense fluorescence.
- Although the **red fluorescent FluoSpheres beads** are maximally excited at 580 nm, the excitation band is broad enough so that the beads emit well at 605 nm, even when excited at 488 nm. Our **orange and red/orange fluorescent FluoSpheres beads** have excitation maxima of 540 and 565 nm, respectively.
- Our **nile red fluorescent FluoSpheres beads** have broad excitation and emission bandwidths, making them compatible with fluorescein, rhodamine and Texas Red® optical filter sets.
- Our **crimson and dark red fluorescent FluoSpheres beads** are efficiently excited by the 633 nm spectral line of the He-Ne laser. Although the dark red fluorescent particles are significantly less fluorescent than the crimson fluorescent particles, they fluoresce at wavelengths that are longer and clearly distinguishable from those of the crimson fluorescent particles.
- Our **far red fluorescent FluoSpheres beads** with excitation/emission maxima of 690/720 nm are compatible with diode lasers — inexpensive excitation sources that are increasingly being used in fluorescence instrumentation.[1] These far red fluorescent beads may also prove useful for making direct fluorescence measurements in autofluorescent materials such as blood, plant tissues and marine organisms.
- Our **infrared fluorescent FluoSpheres beads** with excitation/emission maxima of 715/755 nm are the longest-wavelength fluorescent microspheres currently available. These beads absorb and emit at wavelengths at which most tissues are almost optically transparent.

We have found our FluoSpheres beads to be many times brighter than other available fluorescent microsphere products. These comparisons were made using identical particle sizes and concentrations and exciting both samples at their excitation maximum. Table 2 shows the approximate number of unquenched fluorescein equivalents in our yellow-green fluorescent FluoSpheres beads. The intensity of the beads is sufficient to allow visualization of single particles, even for our smallest microspheres, which appear as point sources. Moreover, aqueous suspensions of FluoSpheres beads do not fade significantly when illuminated by a 250-watt xenon-arc lamp for 30 minutes. Indeed, most of our FluoSpheres beads show little or no photobleaching, even when excited with the intense illumination required for fluorescence microscopy.

Although some of our FluoSpheres beads are available in limited sizes and surface functions, we will prepare custom

orders upon request. FluoSpheres beads can also be prepared with intensities that are *lower* than those of our regular selection, a desirable feature in some multicolor applications.

Sizes

To meet the diverse needs of our customers, we offer FluoSpheres beads in a variety of sizes (Table 1). The smallest microspheres are currently about 0.02 µm in diameter, with a coefficient of variation (CV) of about 20%, as determined by electron micro-scopy. The size uniformity improves with increasing size, with the CV decreasing from 5% for 0.1 µm FluoSpheres beads to ~1% for those with 10–15 µm diameters. The sizes specified in the product names are nominal bead diameters; because of batch variation in the undyed microspheres, the actual mean diameters shown on the product labels may differ from the nominal diameters, especially for the smaller microspheres. Because of their small size, 0.02–0.04 µm microspheres are transparent to light in aqueous suspensions and behave very much like true solutions.

Surface Functional Groups

We prepare FluoSpheres beads with four different surface functional groups, making them compatible with a variety of conjugation strategies. Our fluorescent dyes have negligible

Table 1. *Summary of Molecular Probes' FluoSpheres fluorescent microspheres.**

Microspheres †	0.02 µm	0.04 µm	0.1 µm	0.2 µm	0.5 µm	1.0 µm	2.0 µm	4.0 µm
Carboxylate-Modified Microspheres								
Blue (365/415)	F-8781 10 mL			F-8805 10 mL		F-8814 10 mL	F-8824 2 mL	
Blue (350/440)			F-8797 10 mL			F-8815 10 mL		
Yellow-green (505/515)	F-8787 10 mL	F-8795 1 mL	F-8803 10 mL	F-8811 10 mL	F-8813 10 mL	F-8823 10 mL	F-8827 2 mL	
Nile red (535/575)	F-8784 10 mL					F-8819 10 mL	F-8825 2 mL	
Orange (540/560)		F-8792 1 mL	F-8800 10 mL	F-8809 10 mL		F-8820 10 mL		
Red-orange (565/580)		F-8794 1 mL						
Red (580/605)	F-8786 10 mL	F-8793 1 mL	F-8801 10 mL	F-8810 10 mL	F-8812 10 mL	F-8821 10 mL	F-8826 2 mL	
Crimson (625/645)	F-8782 2 mL			F-8806 2 mL		F-8816 2 mL		
Dark red (660/680)	F-8783 2 mL	F-8789 1 mL		F-8807 2 mL				
Far red (690/720)			F-8798 1 mL					
Infrared (715/755)		F-8791 0.4 mL	F-8799 1 mL					
Sulfate Microspheres								
Blue (365/415)						F-8849 10 mL		F-8854 2 mL
Yellow-green (505/515)	F-8845 10 mL			F-8848 10 mL		F-8852 10 mL	F-8853 2 mL	F-8859 2 mL
Red (580/605)						F-8851 10 mL		F-8858 2 mL
Aldehyde-Sulfate Microspheres								
Yellow-green (505/515)	F-8760 10 mL					F-8762 10 mL		
Amine-Modified Microspheres								
Yellow-green (505/515)				F-8764 5 mL		F-8765 5 mL		
Red (580/605)				F-8763 5 mL				

***** FluoSpheres beads are supplied as aqueous suspensions containing 2% solids, except for the 0.04 µm microspheres, which are supplied as aqueous suspensions containing 5% solids. All sizes fall within a narrow range. Sizes indicated are nominal and may vary from batch to batch. Actual sizes, as determined by electron microscopy, are specified on the product labels. † Approximate fluorescence excitation and emission in nm are indicated in parentheses.

effect on the surface properties of the polystyrene beads or on their protein adsorption. We caution, however, that the surface properties have an important role in the functional utility of the microspheres; we cannot guarantee the suitability of a particular bead type for all applications.

- **Carboxylate-modified FluoSpheres beads** have pendent carboxylic acids, making them suitable for covalent coupling of proteins and other amine-containing biomolecules using water-soluble carbodiimide reagents such as EDAC (E-2247). In order to both decrease nonspecific binding and provide additional functional groups for conjugation, we use carboxylate-modified beads that have a high density of carboxylic acids on their surface.
- **Sulfate FluoSpheres beads** are relatively hydrophobic particles that will passively adsorb almost any protein, including BSA, IgG, avidin and streptavidin.
- **Aldehyde-sulfate FluoSpheres beads**, which are sulfate microspheres that have been modified to add surface aldehyde groups, are designed to react with proteins and other amines under very mild conditions.
- **Amine-modified FluoSpheres beads** can be coupled to a wide variety of amine-reactive molecules, including succinimidyl esters and isothiocyanates of haptens and drugs or carboxylic acids of proteins, using a water-soluble carbodiimide. The amine surface groups can also be reacted with SPDP (S-1531) to yield (after reduction) microspheres with sulfhydryl groups.

Detailed information on the surface properties of FluoSpheres beads is given in our information sheet "Working with FluoSpheres® Fluorescent Microspheres" (MP 05001), available at our Web site (www.probes.com/media/PIS/MP05001.pdf) or from our Technical Assistance Department.

Biotin-, Avidin- and Protein A–Labeled Microspheres

Molecular Probes offers fluorescent and nonfluorescent biotin-, NeutrAvidin-, streptavidin- and protein A–labeled FluoSpheres beads (Table 3), which can be used to improve the sensitivity of flow cytometry applications and immunodiagnostic assays. They may also be useful as tracers that can be detected with standard avidin/streptavidin enzyme-mediated methods. NeutrAvidin biotin-binding protein is a form of avidin that has been processed to remove carbohydrates and lower the isoelectric point. The resulting near-neutral protein has significantly less nonspecific binding than conventional avidin.

Protein- and other macromolecule-labeled microspheres have hydrophobic regions that may cause them to bind to non-target surfaces in some applications. BlockAid™ blocking solution (B-10710) is designed to reduce nonspecific binding of our streptavidin-, NeutrAvidin-, biotin- and protein A–labeled FluoSpheres microspheres. In flow cytometry applications, we find BlockAid blocking solution reduces nonspecific binding of protein-labeled microspheres better than commercially available blocking solutions or "home-made" blocking solutions described in the scientific literature. We expect BlockAid blocking solution to be useful for preventing the nonspecific binding of protein-coated or other macromolecule-coated microspheres in a variety of flow cytometry and microscopy applications. BlockAid blocking solution is available in a 50 mL unit size.

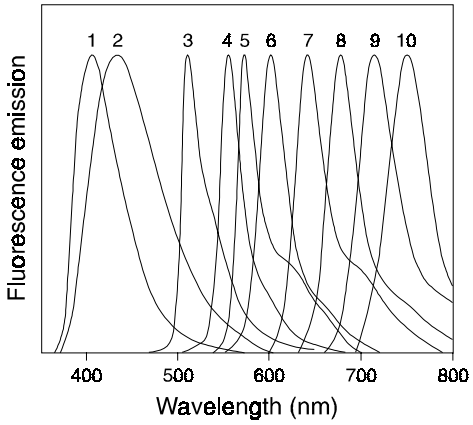

Figure 1. *Normalized fluorescence emission spectra of our FluoSpheres beads, named according to their excitation/emission maxima (nm): 1) blue (365/415), 2) blue (350/440), 3) yellow-green (505/515), 4) orange (540/560), 5) red-orange (565/580), 6) red (580/605), 7) crimson (625/645), 8) dark red (660/680), 9) far red (690/720) and 10) infrared (715/755) FluoSpheres beads.*

Custom FluoSpheres Beads

Molecular Probes can prepare custom-dyed or custom-coated microspheres for your application. Contact our Custom and Bulk Sales Department for further information.

Materials, Storage and Handling

All FluoSpheres products should be stored at 4°C, protected from light. DO NOT FREEZE. Before sampling, mix well by sonication, vigorous shaking or vortex mixing. The microspheres are stable for at least one year, provided recommended storage conditions are strictly observed.

Our standard FluoSpheres beads are supplied as suspensions (2% solids) in water plus 2 mM sodium azide. Suspensions of 2.0 µm and 4.0 µm beads contain, in addition, 0.02% Tween® 20. The 0.04 µm FluoSpheres beads are provided at 5% solids in water (without sodium azide). The unit sizes are indicated in Table 1. The biotin- and NeutrAvidin-labeled FluoSpheres beads are supplied as 1% solids in 50 mM sodium phosphate, 50 mM NaCl, pH 7.5 plus 0.02% Tween 20 and 2–5 mM sodium azide. The streptavidin- and protein A–labeled FluoSpheres beads are supplied as 0.5% solids in 50 mM sodium phosphate, 50 mM NaCl, pH 7.5 plus 2–5 mM sodium azide. The unit sizes for

Table 2. *Fluorescein equivalents in our yellow-green fluorescent FluoSpheres beads.*

Microsphere Diameter (µm)	Fluorescein Equivalents per Microsphere
0.02	1.8×10^2
0.04	3.5×10^2
0.1	7.4×10^3
0.2	1.1×10^5
0.5	2.0×10^6
1.0	1.3×10^7
2.0	3.1×10^7
10	1.1×10^{10}
15	3.7×10^{10}

*Table 3. Summary of biotin-, streptavidin, NeutrAvidin- and protein A–labeled FluoSpheres microspheres.**

Microspheres †	0.04 µm	0.2 µm	1.0 µm
Biotin-Labeled Microspheres			
Yellow-green (505/515)	F-8766 0.4 mL	F-8767 0.4 mL	F-8768 0.4 mL
Nonfluorescent			F-8769 0.4 mL
Streptavidin-Labeled Microspheres			
Yellow-green (505/515)	F-8780 0.4 mL		
NeutrAvidin-Labeled Microspheres			
Yellow-green (505/515)	F-8771 0.4 mL	F-8774 0.4 mL	F-8776 0.4 mL
Red (580/605)	F-8770 0.4 mL		F-8775 0.4 mL
Nonfluorescent	F-8772 0.4 mL		F-8777 0.4 mL
Protein A–Labeled Microspheres			
Yellow-green (505/515)	F-8778 0.4 mL		F-8779 0.4 mL

***** Biotin- and NeutrAvidin-labeled FluoSpheres beads are supplied as aqueous suspensions containing 1% solids and 0.02% Tween® 20; the streptavidin- and protein A–labeled FluoSpheres beads are supplied as aqueous suspensions containing 0.5% solids without surfactant. All sizes fall within a narrow range as discussed in the text. Sizes indicated in the above tables are nominal and may vary from batch to batch. Actual sizes, as determined by electron microscopy, are specified on the product label. † Approximate fluorescence excitation and emission in nm are indicated in parentheses.

the biotin-, NeutrAvidin-, streptavidin- and protein A–labeled FluoSpheres products are indicated in Table 4.

The number of microspheres per mL of suspension may be determined from the following equation:

$$\text{Number of microspheres/ mL} = \frac{6C \times 10^{12}}{\rho \times \pi \times \phi^3}$$

Where: C = concentration of suspended beads in g/mL (0.02 g/mL for a 2% suspension)
ϕ = diameter of microspheres in µm
ρ = density of polymer in g/mL (1.05 for polystyrene)

For example, for a 2% suspension of 10 µm polystyrene beads:

$$\text{Number of microspheres/mL} = \frac{6(0.02) \times 10^{12}}{1.05 \times \pi \times (10)^3} = 3.6 \times 10^7$$

Fluorescent Microsphere Starter Kits

For first time users, we offer several fluorescent microsphere starter kits:

- **FluoSpheres Fluorescent Color Kit** (F-10720) consists of 1 mL samples of yellow-green, orange, red and dark red fluorescent carboxylate-modified 0.04 µm FluoSpheres beads packaged as high-density, azide-free suspensions for microinjection.
- **FluoSpheres Size Kits** contain 1 mL samples of carboxylate-modified FluoSpheres beads in 0.02, 0.1, 0.2, 0.5, 1.0 and 2.0 µm sizes and are available in yellow-green (F-8888) or red (F-8887) fluorescent colors.
- **FluoSpheres Blood Flow Determination Fluorescent Color Kits** provide several different fluorescent colors of our 10 µm (F-8890) or 15 µm (F-8891, F-8892) FluoSpheres polystyrene microspheres.

Applications

Fluorescent microspheres have been used as markers for cellular antigens,[2] as retrograde neuronal tracers,[3] as microinjectable cell tracers [4,5] and as standardization reagents for flow cytometry.[6] Moreover, they have been employed to investigate phagocytic processes [7-9] and to determine blood flow in tissues.[10] Because of their high fluorescence intensity, FluoSpheres beads should be especially suited for the many microsphere-based diagnostic tests that have been developed.[2] The far red excitation microspheres are particularly useful when making direct fluorescence measurements in materials that significantly autofluoresce, such as serum and whole blood. Detecting fluorescence at long wavelengths has the additional benefit of reducing background that arises from sample auto-fluorescence and from Rayleigh and Raman scattering interference.

Cell-Surface Antigen Detection

The bright fluorescence of our FluoSpheres beads makes them ideally suited for detecting low-density receptors on cell surfaces.[11,12] Fluorescent microspheres coupled to goat anti-rabbit antibodies were shown to bind specifically to red blood cells and lymphocytes that were previously sensitized with rabbit antibodies to cell-surface antigens.[13] Antibody-coated fluorescent microspheres have also been employed to detect donor erythrocytes in patients who had received allogenic bone marrow transplants.[14] Using similar techniques, researchers have used three sizes of fluorescent microspheres to simultaneously detect three different *Candida albicans* antigens.[15]

Neuronal Retrograde Tracers

Katz was the first to use fluorescent microspheres as a neuronal tracer, demonstrating that rhodamine-labeled microspheres could be retrogradely transported.[16] It has since been shown that similar green fluorescent microspheres undergo retrograde transport,[17] although not as readily as rhodamine-labeled microspheres in these experiments. Using fluorescent microspheres in these applications has the following advantages:

- Polystyrene microspheres are not cytotoxic.
- They diffuse minimally from the injection site.
- They persist for extraordinarily long periods in nerve cells.

The intensity of labeling of neuronal perikarya in rats has been reported to be undiminished one year after injection.[18] Although the exact mechanism of transport is not completely understood, the transport process can apparently be facilitated by using high concentrations of particles with small diameters (<0.05 µm) and high negative surface-charge densities.[19,20] We formerly listed 0.03 µm carboxylate-modified microspheres for use in retrograde neuronal tracing studies. Because of conflict-

ing reports on the utility of these products for retrograde tracing, we no longer recommend these products specifically for that application. However, we still make these carboxylate-modified microspheres available to researchers for use as microinjectable tracers or for possible use in retrograde tracing studies or other applications. (The nominal size of these microspheres, however, is now listed as 0.04 µm.) Unlike our other fluorescent microspheres, most of which are sold in suspensions containing 2% solids and 2 mM sodium azide as a preservative, these products are now sold as 5% solids, without preservatives, to facilitate their use in these specialized applications. Our biotinylated fluorescent and nonfluorescent microspheres may permit detection by fluorescence followed by other ultrastructural techniques. Fluorescent microspheres have also been detected by electron microscopy by using potassium permanganate for negative contrast.[21]

Tracers for Phagocytosis

It has been shown that 0.6–2.0 µm fluorescent microspheres can be used to investigate phagocytic processes in rat neutrophils,[22] human trabecular meshwork cells,[23] mouse peritoneal macrophages [24] and human polymorphonuclear leukocytes.[25] Analysis of phagocytized particles has been carried out by quantitative flow cytometry.[26,27] Because of their low nonspecific binding, carboxylate-modified microspheres appear to be best for phagocytic applications. Various opsonizing agents such as fetal calf serum or rabbit serum have been used to facilitate phagocytosis.

Sensitive Diagnostic Reagents

Several successful commercial diagnostic tests that employ nonfluorescent microspheres already exist, including two for β-HCG (Unipath's Clearblue Easy™ and Tambrand's First Response™). Fluorescent microspheres can be used in most, if not all of the major microsphere-based diagnostic test systems presently in use, including latex-agglutination tests, filter-separation tests, particle-capture ELISA methods and two-particle sandwich techniques. Fluorescent microspheres provide quantitative, as well as qualitative results, and are potentially more sensitive than colorimetric methods.

Blood Flow Measurements

Molecular Probes has developed a range of microsphere products specifically for fluorescence-based (as opposed to radioisotope-based) measurements of regional blood flow in tissues. Our FluoSpheres products for regional blood flow determination are described in a separate information sheet "FluoSpheres® Fluorescent Microspheres for Blood Flow Determination" (MP 08829), which is available at our Web site (www.probes.com/media/PIS/MP08829.pdf) or from our Technical Assistance Department. Our FluoSpheres Blood Flow Determination Fluorescent Color Kits contain either 10 µm or 15 µm microspheres in seven fluorescent colors or 15 µm microspheres in five fluorescent colors.

TransFluoSpheres® Beads

Molecular Probes has a line of fluorescent microspheres that incorporate a series of two or more proprietary dyes that are carefully chosen to allow efficient energy transfer between the dyes. This patented technology produces microspheres that exhibit extremely large Stokes shifts. More information can be found in the information sheet entitled "TransFluoSpheres® Fluorescent Microspheres" (MP 07186), available at our Web site (www.probes.com/media/PIS/MP07186.pdf) or by request from our Technical Assistance Department.

References

1. Cytometry 15, 267 (1994); **2.** *Flow Cytometry and Sorting, 2nd Edition,* M.R. Melamed *et al*., Eds., pp. 367B380, Wiley-Liss, Inc. (1990); **3.** Nature 310, 498 (1984); **4.** Cell Motil Cytoskeleton 8, 293 (1987); **5.** Dev Growth Differ 28, 461 (1986); **6.** Clin Immunol Immunopathol 55, 187 (1990); **7.** Science 215, 64 (1982); **8.** J Leukoc Biol 41, 95 (1987); **9.** J Leukoc Biol 43, 143 (1988); **10.** J Auton Nerv Syst 30, 159 (1990); **11.** Biochem Biophys Res Commun 188, 1223 (1991); **12.** Am J Physiol 255, C452 (1988); **13.** J Cell Biol 64, 75 (1974); **14.** Br J Haematol 72, 239 (1989); **15.** J Immunol Methods 116, 213 (1989); **16.** Nature 310, 498 (1984); **17.** Neuroscience 34, 511 (1990); **18.** Brain Res 524, 339 (1990); **19.** J Neurosci Methods 29, 1 (1989); **20.** Brain Res 522, 90 (1990); **21.** J Neurosci Methods 23, 181 (1988); **22.** Biochem J 266, 669 (1990); **23.** Invest Ophthalmol Vis Sci 30, 2499 (1989); **24.** Anal Biochem 152, 167 (1986); **25.** J Immunol Methods 88, 175 (1986); **26.** Science 215, 64 (1982); **27.** Cytometry 12, 677 (1991).

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

Cat #	Product Name	Unit Size
B-10710	BlockAid™ blocking solution *for use with microspheres*	50 mL
F-8890	FluoSpheres® Blood Flow Determination Fluorescent Color Kit #1, polystyrene microspheres, 10 µm *seven colors, 10 mL each* *3.6 x10^6 beads/mL*	1 kit
F-8891	FluoSpheres® Blood Flow Determination Fluorescent Color Kit #2, polystyrene microspheres, 15 µm *seven colors, 10 mL each* *1.0 x10^6 beads/mL*	1 kit
F-8892	FluoSpheres® Blood Flow Determination Fluorescent Color Kit #3, polystyrene microspheres, 15 µm *five colors, 10 mL each* *1.0 x10^6 beads/mL*	1 kit
F-8893	FluoSpheres® Blood Flow Determination Fluorescent Color Kit #4, polystyrene microspheres, 15 µm *five colors, 2 mL each* *1.0 x10^6 beads/mL*	1 kit
F-10720	FluoSpheres® Fluorescent Color Kit, carboxylate-modified microspheres, 0.04 µm *four colors, 1 mL each* *5% solids, azide free*	1 kit
F-8887	FluoSpheres® Size Kit #1, carboxylate-modified microspheres, red fluorescent (580/605) *six sizes, 1 mL each* *2% solids*	1 kit
F-8888	FluoSpheres® Size Kit #2, carboxylate-modified microspheres, yellow-green fluorescent (505/515) *six sizes, 1 mL each* *2% solids*	1 kit

For listing of available carboxylate-modified, sulfate, aldehyde-sulfate and amine-modified FluoSpheres® microspheres, see Table 1.
For listing of available biotin-, streptavidin-, NeutrAvidin- and Protein A-labeled FluoSpheres® microspheres, see Table 3.

Contact Information

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

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