

TransFluoSpheres® Fluorescent Microspheres

Quick Facts

Storage upon receipt:

- 2–6°C
- Do not freeze
- Protect from light

Ex/Em: See Table 1

Note: Mix well before use

Introduction

TransFluoSpheres® fluorescent microspheres incorporate a series of two or more proprietary dyes that are carefully chosen to allow excited-state energy transfer between dyes, thus producing fluorescent microspheres with extremely large Stokes shifts (the separation between excitation and emission maxima).¹ Each microsphere contains a dye with an excitation peak that maximally overlaps the spectral output of a commonly used excitation source (for example, the 488 nm spectral line of the argon-ion laser). In addition, the microsphere contains one or more longer-wavelength dyes that are carefully chosen to create a relay series that can efficiently transfer the energy from the initially excited dye to the longest-wavelength acceptor dye. The proprietary dyes used in the TransFluoSpheres® beads are optimally loaded to ensure that the excitation energy is efficiently transferred from dye to dye so that essentially only the longest-wavelength dye in the series exhibits significant fluorescence. Because these TransFluoSpheres® beads fluoresce at a wavelength that is considerably longer than the excitation wavelength, they provide a signal that can be detected in samples with significant Rayleigh or Raman scattering or with endogenous fluorescent compounds such as bilins, flavins, and certain drugs. Also, the large Stokes shifts exhibited by the TransFluoSpheres® beads allow the use of broad-band filters, both to excite the sample and to detect the emission, resulting in a greater fluorescent signal.

TransFluoSpheres® beads, which are manufactured using high-quality, carboxylate-modified polystyrene beads, are loaded with dyes by a process that does not significantly modify the surface properties of the beads. The beads are available in 0.04 µm, 0.1 µm, and 1.0 µm diameters. We also offer TransFluoSpheres® beads covalently labeled with streptavidin or NeutrAvidin™ biotin-binding protein, which provide researchers with valuable tools for improving the sensitivity of flow cytometry applications

Table 1. Summary of Molecular Probes' TransFluoSpheres® fluorescent microspheres.

Excitation/Emission (nm)	Size		
	0.04 µm	0.1 µm	1.0 µm
488/560	T8864 0.5 mL	T8872 0.5 mL	T8880 0.5 mL
488/605 NeutrAvidin™-labeled	T8860 0.4 mL	T8861 0.4 mL	
488/645	T8867 0.5 mL		T8883 0.5 mL
488/645 Streptavidin-labeled	T10711 0.4 mL		
488/685	T8868 0.5 mL	T8876 0.5 mL	
488/720	T8869 0.5 mL		
543/620		T8874 0.5 mL	
633/720	T8870 0.5 mL	T8878 0.5 mL	
633/760 For tracer studies			T13090* 0.5 mL

* Product T13090 has a 2.0 µm diameter.

and immunodiagnostic assays. These microspheres can also serve 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.

Table 1 provides a summary of Molecular Probes' TransFluoSpheres fluorescent microspheres. The table conveniently presents catalog numbers, unit sizes, spectral properties, and bead diameters for the microspheres that we offer.

Colors, Sizes and Surface Characteristics

Spectral Properties

Molecular Probes currently offers TransFluoSpheres® beads that are compatible with two commonly used excitation sources:

- A series of five argon-ion laser-excitabile TransFluoSpheres® beads, all of which have an excitation maximum at 488 nm but emit at different wavelengths: 560 nm, 605 nm, 645 nm, 685 nm, or 720 nm (Figure 1)

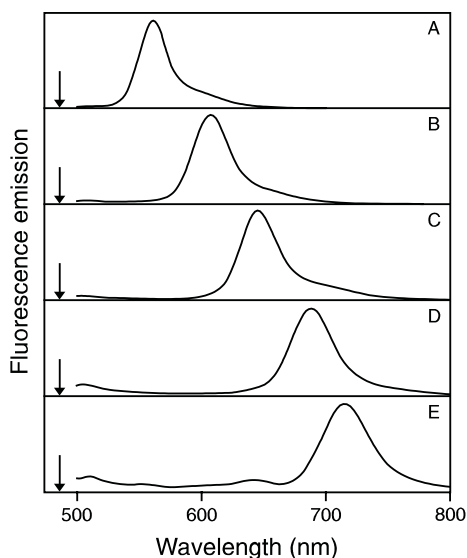


Figure 1. Fluorescence emission spectra of our 488 nm–excitable TransFluoSpheres® beads, named according to their excitation maximum (nm)/emission maximum (nm): A) 488/560, B) 488/605, C) 488/645, D) 488/685, and E) 488/720. The arrow in each spectrum represents the 488 nm spectral line of the argon-ion laser.

- Red He-Ne laser–excitable TransFluoSpheres® beads with excitation/emission maxima of 633/720 nm or 633/760 nm
- Green He-Ne laser–excitable TransFluoSpheres® beads with excitation/emission maxima of 543/620 nm; these TransFluoSpheres® beads are also efficiently excited by a mercury-arc lamp

The series of TransFluoSpheres® beads with 488 nm excitation enables researchers to detect five experimental parameters simultaneously. TransFluoSpheres® beads can also be combined with our more traditional FluoSpheres® beads or with low molecular weight dyes for multicolor detection.

Sizes

TransFluoSpheres® beads are available in four sizes. The smallest size, 0.04 μm diameter, is the size most widely used for neuronal retrograde transport studies. The coefficient of variation of beads in this size group is generally about 20% as determined by electron microscopy. Size uniformity improves with increasing bead diameter. We also offer TransFluoSpheres® beads in 0.1 μm , 1.0 μm , and 2.0 μm diameters. For these sizes, the coefficient of variation decreases to between 1% and 5%.

Surface Functional Groups

TransFluoSpheres® beads are prepared with carboxylate-modified microspheres, which are polystyrene microspheres coated with a hydrophilic polymer containing multiple carboxylic acids. These hydrophilic particles will passively adsorb almost any protein. Our fluorescent dyes have no significant effect on the surface properties of the microspheres and do not appear to affect protein adsorption. In addition, proteins and other amine-containing biomolecules can be covalently coupled to the pendent carboxyl groups of these TransFluoSpheres® beads using water-soluble carbodiimide reagents such as EDAC (available from Molecular Probes, E2247), making these microspheres suitable for a wide range of applications. TransFluoSpheres® beads can be used in the major microsphere-based diagnostic test systems,

as well as in experiments that currently employ standard fluorescent microspheres to detect cell-surface antigens, trace neurons, and study phagocytosis.²⁻⁶ More detailed practical information on the surface properties of fluorescent microspheres is given in our information sheet *Working with FluoSpheres® Fluorescent Microspheres* (MP 05001), which is available at our website (probes.invitrogen.com) or by request from our Technical Service Department.

Avidin-Labeled TransFluoSpheres® Beads

The 488/645 TransFluoSpheres® beads, 0.04 μm diameter, are available covalently labeled with streptavidin. Two sizes of 488/605 TransFluoSpheres® beads (0.04 and 0.1 μm diameters) are available covalently labeled with NeutrAvidin™ biotin-binding protein, a form of avidin 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 (B10710) is designed to reduce nonspecific binding of our streptavidin-, NeutrAvidin™-, biotin-, and protein A–labeled FluoSpheres® and TransFluoSpheres® 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. BlockAid™ blocking solution is 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.

TransFluoSpheres® for Blood Flow Measurement in Live Animals

Molecular Probes' 2.0 μm TransFluoSpheres® polystyrene microspheres (633/760) (T13090) have been successfully used to measure regional retinal and choroidal blood circulation in live rhesus monkeys.⁷ In the past, researchers using fluorescent microspheres to measure regional blood flow in animals had to sacrifice the animal subjects. Now, with our new 2.0 μm TransFluoSpheres® beads, which emit in the infrared region of the spectrum, the circulation in superficial blood vessels can be detected in live animals.⁸

Custom TransFluoSpheres® Beads

If your wavelength or bead-size requirements are not met by our current stock products or you desire beads coated with other proteins or with biotin, we invite your inquiry. Please contact our Custom and Bulk Sales Department for further information.

Materials, Storage, and Handling

All TransFluoSpheres® bead suspensions should be stored at 2–6°C, protected from light. DO NOT FREEZE. Before sampling, mix well by sonication, vigorous shaking, or vortex mixing. The TransFluoSpheres® fluorescent microspheres are stable for at least one year, provided recommended storage conditions are strictly observed.

Our standard TransFluoSpheres® beads are supplied in unit sizes of 0.5 mL as suspensions (2% solids) in water plus 2 mM sodium azide. Our TransFluoSpheres® microspheres (633/760) for tracer studies are supplied in a unit size of 0.5 mL at 1.0×10^9 beads/mL (~0.4% solids) as a suspension in water plus 0.02% Tween® 20 and 2 mM sodium azide or 0.02% thimerosal. Streptavidin-labeled TransFluoSpheres® beads are supplied in unit sizes of 0.4 mL as a suspension (0.5% solids) in 50 mM sodium phosphate, 50 mM NaCl, pH 7.5, plus 5 mM sodium azide. NeutrAvidin™-labeled TransFluoSpheres® beads are supplied in unit sizes of 0.4 mL as suspensions (1% solids) in 50 mM sodium phosphate, 50 mM NaCl, pH 7.5, plus 0.02% Tween 20, and 2–5 mM sodium azide.

Applications

Standard fluorescent microspheres have been used in a wide variety of experimental and diagnostic procedures, such as detecting cell-surface antigens,^{2,3} tracing neurons,^{4,5} and studying phagocytosis.⁶ Our novel TransFluoSpheres® beads should prove superior in many of these applications because their large Stokes shifts eliminate many of the problems related to cellular autofluorescence and other background sources. TransFluoSpheres® beads can also be used in most, if not all, of the major microsphere-based diagnostic test systems presently in use, including microsphere-agglutination⁹ and filter-separation tests, particle-capture ELISA methods, and two-particle sandwich techniques.

References

1. U.S. Patent No. 5,326,692; 2. *Biophys J* 65, 2396 (1993); 3. *J Membrane Biol* 135, 82 (1993); 4. *J Neurosci* 13, 5082 (1993); 5. *Brain Res* 630, 115 (1993); 6. *Cytometry* 13, 423 (1992); 7. *Ophthalmic Surg Lasers* 28, 937 (1997); 8. Visualizing submicron particles in blood flow in the eye of an animal is covered by U.S. Patent No. 5,437,274, owned by Louisiana State University. Purchase of these products does not convey a license under this patent; 9. Hemmila, I.A., *Applications of Fluorescence in Immunoassays*, John Wiley and Sons (1991) pp. 49, 160–162.

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

For listing of available TransFluoSpheres® Fluorescent Microspheres, see Table 1, or visit our website (probes.invitrogen.com).

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 Paisley, United Kingdom. All others should contact our Technical Service Department in Eugene, Oregon.

Please visit our website — probes.invitrogen.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 • probesorder@invitrogen.com

Toll-Free Ordering for USA:

Order Phone: (800) 438-2209 • Order Fax: (800) 438-0228

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

Phone: (541) 335-0353 • Toll-Free (800) 438-2209

Fax: (541) 335-0238 • probestech@invitrogen.com

Invitrogen European Headquarters

Invitrogen, Ltd.

3 Fountain Drive

Inchinnan Business Park

Paisley PA4 9RF, UK

Phone: +44 (0) 141 814 6100 • Fax: +44 (0) 141 814 6260

Email: euroinfo@invitrogen.com

Technical Services: eurotech@invitrogen.com

Molecular Probes products are high-quality reagents and materials intended for research purposes only. These products must be used by, or directly under the supervision of, a technically qualified individual experienced in handling potentially hazardous chemicals. Please read the Material Safety Data Sheet provided for each product; other regulatory considerations may apply.

Limited Use Label License

For research use only. Not intended for any animal or human therapeutic or diagnostic use. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) to not transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. Invitrogen Corporation will not assert a claim against the buyer of infringement of the above patents based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851 Willow Creek Road, Eugene, OR 97402. Tel: (541) 465-8300. Fax: (541) 335-0504.

Several Molecular Probes products and product applications are covered by U.S. and foreign patents and patents pending. All names containing the designation ® are registered with the U.S. Patent and Trademark Office.

Copyright 2005, Molecular Probes, Inc. All rights reserved. This information is subject to change without notice.