

## Internally labeled polymer microspheres

### Product Information

Product name cat.number	Nominal Diameter	Mean Diameter	Size (CV) Uniformity	Number/g or Density
Green fluorescent microspheres, (470/508 nm) FP-BX3410, 15 ml	0,03 µm	0,028 µm	<20%	8,3 x 10 <sup>14</sup>
Green fluorescent microspheres, (470/508 nm) FP-BT9880, 15 ml	0,10 µm	0,10 µm	<10%	1,8 x 10 <sup>13</sup>
Green fluorescent microspheres, (470/508 nm) FP-CJ2680, 15 ml	0,20 µm	0,20 µm	<10%	2,3 x 10 <sup>12</sup>
Green fluorescent microspheres, (470/508 nm) FP-BZ5400, 15 ml	0,40 µm	0,39 µm	<5%	3,1 x 10 <sup>11</sup>
Green fluorescent microspheres, (470/508 nm) FP-M1949B, 10 ml (1% solids)	1,0 µm	1,0 µm	<5%	1.05g/cm <sup>3</sup>
Red fluorescent microspheres, (542/612 nm) FP-GV3370, 15 ml	0,05 µm	0,049 µm	<15%	1,5 x 10 <sup>14</sup>
Red fluorescent microspheres, (542/612 nm) FP-BT9980, 15 ml	0,10 µm	0, 10 µm	<10%	1,8 x 10 <sup>13</sup>
Red fluorescent microspheres, (542/612 nm) FP-CJ2740, 15 ml	0,40 µm	0,40 µm	<5%	2,8 x 10 <sup>11</sup>
Red fluorescent microspheres, (575/600 nm) FP-CA8790, 1 g	70 µm	68 µm		5,8 x 10 <sup>6</sup>

**Storage:** +2° to 8°C (H)

**DO NOT FREEZE**

### Introduction

From their serendipitous development in 1947<sup>1</sup>, microspheres have enjoyed respectable ‘careers’ in a variety of disciplines. Within the field of biomedicine alone, we have witnessed the evolution of microsphere applications from the earliest latex agglutination tests<sup>2</sup> to today’s sophisticated multiplexed assays<sup>3,35</sup>.

The proliferation of microsphere-based tests and assays is owed in no small part to the versatility of the beads, themselves. Microspheres are available with a variety of functionalized surfaces, densities and special properties (e.g. magnetic). The association of colored dyes or fluorophores adds a further level of flexibility. In fact, the incorporation of fluorophores has become a particularly important feature for assay development, conferring

FT-BZ5400

benefits such as multiplexing capabilities<sup>35</sup> and signal enhancement, and serving as a replacement for radioactive labels<sup>4</sup>.

Microspheres are commonly dyed after synthesis, through dye entrapment (internal labeling) or surface attachment (external labeling)<sup>29,30</sup>. Dye or fluorophore diffusion / entrapment involves the swelling of polymeric microspheres in an organic solvent / dye solution. The water-insoluble dye diffuses into the polymer matrix, and is entrapped when the solvent is removed from the microspheres (through evaporation or transfer to an aqueous phase).

The great majority of FluoProbes' microspheres are internally labeled, which affords many benefits, including:

- availability of surface groups for coupling reactions;
- photostability, protection of fluorophore from photobleaching;
- larger selection of dyes;
- wide range of sizes available, ~0.02µm+ (no upper limit);
- greater dye loading / brighter microspheres;

Figure 1 illustrates the dye loading that is possible using the diffusion method. For colored (non-fluorescent) dyes, an amount of dye equal to approximately 10-40% of the bead weight may be entrapped within each, and up to approximately 1% of the bead weight for fluorophores (a lesser amount due to problems associated with intermolecular interaction of fluorophore molecules [fluorescence quenching]).

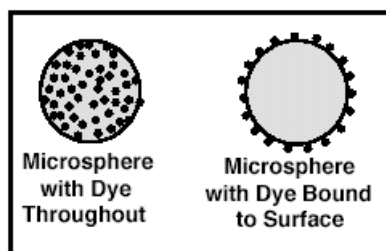


Figure 1

Microspheres may also be externally labeled with dyes (generally fluorophores), as are some of the FluoProbes Flow Cytometry products (see VeriFMC™ products). Surface attachment of fluorophores offers other unique benefits, such as:

- environmental responsiveness of the dye;
- spectral retention (spectra are much like those of free dye);
- useful where particles will be used in solvent and will swell; dye will not escape.

The microspheres are made of polystyrene, which has a density of 1.06g/cm<sup>3</sup> and a refractive index of 1,59 at 589nm. The aqueous suspensions are packaged as 1% solids in a multicomponent dispersing system which prevents clumping and aids in dispersion.

## Directions for use

Expiration date: >24 months

### Guidelines for use –

Polymer microspheres > 0.5 µm in suspension will sediment over time.

The particles must be thoroughly dispersed and resuspended before use. The suspension can be resuspended by gently inverting, rolling or swirling the bottle, followed by a brief (15 sec.) immersion in a low power ultrasonic bath. The microspheres can be dispensed using the dropper tip or by using a clean pipette. A clean pipette can be used to take samples from the bottle. Shaking the bottle is not recommended, as the product may foam.

FT-BZ5400

Sonication after resuspension is recommended to de-gas and break up temporary agglomerates. For applications that require the microspheres to be suspended for an extended period of time, a clean magnetic stir bar may be used.

### **Applications**

Many applications will require a more dilute suspension. The end-user may dilute these products with particle-free, deionized water. If the dilution is too great, or there appears to be some aggregation of the particles, a non-ionic or anionic surfactant solution should suffice. Generally, this will not be needed. We recommend dilution immediately prior to use as the diluted suspensions may not be stable for long periods of time. These products should not be suspended in organic solvents.

The products are intended for research and testing applications only. They are not suitable for calibration or applications that require NIST traceable mean diameters. The mean diameter of these products was determined by either photon correlation spectroscopy (PCS), laser diffraction, or optical microscopy. These products have an extremely wide range of applications. Protocols may be found in the literature. Also please feel free to contact us.

### **Dilution**

Most particle suspensions are suitable for dilution and do not require additional surfactant/dispersant, however, the diluted suspensions should be used immediately as the stability may be affected.

- 1- Calculate the quantity of microspheres needed based on desired final concentration and quantity.
- 2- Resuspend the original microsphere suspension.
- 3- Sample immediately into clean container.
- 4- Add filtered deionized water to desired amount.

### **Suspending dry particles**

This procedure outlines the steps necessary to put a dry powder into suspension.

- 1- Wet the dry particles with a 1% surfactant solution (anionic or non-ionic, i.e., Tween 20 or Triton X100) or an alcohol such as methanol or ethanol.
- 2- Add filtered water to the desired amount.

### **Drying a suspension**

Drying a suspension to achieve a dry powder is not recommended. The microspheres may form permanent aggregates and may be aerosolized creating an inhalation hazard.

### **Dissolving polystyrene microspheres**

In general, aromatic hydrocarbons will dissolve polystyrene. Some commonly used solvents for this application are:

Benzene  
Methyl Ethyl Ketone (MEK)  
Toluene

\* MEK and toluene will dissolve polystyrene divinylbenzene (PSDVB) over time.

### **Removing/Reducing additives by ion exchange or dialysis**

These procedures are used to achieve low or surfactant-free suspensions for applications such as aerosol and biotechnology applications. However, removing the surfactant from a suspension may compromise the stability of the product and should be performed immediately prior to use.

#### Ion exchange

This procedure is recommended for removing ionic surfactants from the suspension and surface of the microspheres.

- 1- Obtain mixed bed ion-exchange resin (i.e. mixed bed resin AG501-X8; Bio-Rad)
- 2- For a 15 ml bottle of particles at 1% solids use 3 to 4 g of resin.



## FT-BZ5400

- 3- Wash the resin thoroughly to remove potential contaminants.
  - Wash resin with approximately 200 ml deionized water five times.
  - Allow the resin to settle, and pour off the water
- 4- Add the particle suspension to the resin in a small bottle. Add extra water if needed.
- 5- Roll the mixture for 4 to 6 hours and filter through washed glass wool to remove the resin.  
(alternatively, let the resin settle and pour off the suspension into another clean bottle.)

## Dialysis

This procedure is recommended for removing surfactants from the suspension (but not from the microsphere surface).

- 1- Wash the dialysis tubing (i.e., CelluSep T3 12-14000 molecular weight cut-off) thoroughly with deionized water and place it in a container of deionized water, submerged.
- 2- Keep refrigerated for storage.
- 3- When ready to use, cut off the desired length of tubing.
- 4- Place a clamp on one end or tie it off.
- 5- Fill about half full with the microsphere suspension.
- 6- Clamp or tie the top end and place in the container of deionized water with at least 10 to 20 times the volume of the latex.
- 7- Roll or stir the contents of the container.
- 8- Allow to dialyze for at least 4 hours.
- 9- Repeat dialysis three times with fresh water.

## Related products

- CelluSep T3 dialysis membrane, [975640](#), 15 m
- Tween 20, [15874A](#), 1 L

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**FT-BZ5400**

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## Ordering information

Catalog size quantities and prices may be found at <http://www.fluoprobes.com>

Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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