

## PS-Speck™ Microscope Point Source Kit (P7220)

### Quick Facts

#### Storage upon receipt:

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

### Introduction

Molecular Probes' PS-Speck™ Microscope Point Source Kit (P7220) contains four different colors of fluorescent microspheres; each microsphere has a diameter of  $0.175 \pm 0.005 \mu\text{m}$ . This exceptionally small diameter coefficient of variation makes the PS-Speck microspheres ideal as uniform, subresolution fluorescent point sources for calibrating instrumental optics, especially in three-dimensional imaging applications. This kit's four ready-to-use 1 mL suspensions contain bright, monodisperse microspheres with excitation/emission wavelengths of 360 nm/440 nm (blue), 505 nm/515 nm (green), 540 nm/560 nm (orange) and 633 nm/660 nm (deep red). The PS-Speck Kit also includes a mounting protocol for the user's convenience and sufficient mounting medium to prepare about 100 slides of each.

### Materials

#### Contents

The PS-Speck Kit contains four aqueous suspensions of fluorescent microspheres, including:

- **Blue fluorescent microspheres** (Component A), 360/440 nm excitation/emission maxima, 1 mL suspension
- **Green fluorescent microspheres** (Component B), 505/515 nm excitation/emission maxima, 1 mL suspension
- **Orange fluorescent microspheres** (Component C), 540/560 nm excitation/emission maxima, 1 mL suspension
- **Deep red fluorescent microspheres** (Component D), 633/660 nm excitation/emission maxima, 1 mL suspension

Each suspension has a density of  $\sim 3 \times 10^9$  beads/mL and provides enough material to mount about 100 slides. In addition, the PS-Speck Kit includes 5 mL of mounting medium (Component E) and a mounting protocol (see *External Fluorescence Microscopy Standards*). Note that this mounting medium does not harden or gel.

### Storage and Handling

Upon receipt, the kit components should be stored refrigerated at 4°C and protected from light. **DO NOT FREEZE.**

The aqueous suspensions of microspheres contain 2 mM sodium azide to inhibit bacterial growth and are provided at a concentration appropriate for most applications. The particle concentration can be increased or the solution exchanged by centrifuging the suspension at about  $12,000 \times g$  for 2 minutes in a microcentrifuge. The particle concentration can be reduced by diluting with water.

The PS-Speck microspheres should appear as monodisperse singlet particles when attached to a surface (see *External Fluorescence Microscopy Standards*). Note that the microspheres will undergo rapid Brownian motion if they are not attached to some surface. Slides coated with polylysine or protein will allow the beads to attach. Because of their brightness, this rapid motion may cause the microspheres to appear as doublets or distorted in some other fashion.

### Applications

A number of representative applications for PS-Speck microspheres are described below.

#### Internal Fluorescence Microscopy Standards

PS-Speck microspheres can be incorporated into experimental samples to serve as internal fluorescent standards. Add a small drop ( $<10 \mu\text{L}$  for a standard coverslip-size sample) of one of the PS-Speck suspensions directly to the sample and visualize with a fluorescence microscope equipped with an appropriate filter set.

#### External Fluorescence Microscopy Standards

PS-Speck microspheres can be mounted on a microscope slide and used as an external fluorescence standard. Add a small drop ( $<10 \mu\text{L}$  for a standard coverslip-size sample) to a microscope slide and air dry; protect from dust during drying. We recommend that you use microscope slides etched with rings to make it easy to identify the position of the microspheres once the drop dries. Alternatively, you can make a circle on the bottom of a standard microscope slide with a marker and place the sample drop on the top of the slide within the circle. When the sample is completely dry, add a small drop of mounting medium to cover the spot, place a coverslip on the slide and seal. The mounting medium will remain liquid; thus, the sample distribution may not be permanent. Visualize the mounted standards with a fluorescence microscope equipped with an appropriate filter set.

### **Multi-Wavelength Applications**

It may be desirable to mix the different fluorescent colors of PS-Speck microspheres together and mount the mixture on a single slide. Alternatively, drops of each suspension may be placed on top of each other on a single microscope slide; however, be sure to reduce the quantity of each individual component unless you want to produce a sample with a higher density of microspheres.

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### **Related Products**

Molecular Probes prepares several other standards for fluorescence microscopy. Our InSpeck™ Microscope Image Intensity Calibration Kits provide intensity reference standards for generating calibration curves and evaluating sample brightness. The kits are offered in a choice of five different fluorescent colors, allowing calibrations over a wide range of excitation and emission wavelengths: InSpeck Blue (350/440 nm), InSpeck Green (505/515 nm), InSpeck Orange (540/560 nm), InSpeck Red (580/605 nm) and InSpeck Deep Red (633/660 nm). Each kit includes six separate suspensions of InSpeck fluorescent microspheres with fluorescence intensities covering the range of intensities commonly encountered in microscopy applications, and unstained control beads and mounting medium are also supplied.

The MultiSpeck™ microspheres in our MultiSpeck Multi-spectral Fluorescence Microscopy Standards Kit exhibit three relatively distinct excitation/emission bands — blue, green and red — in a single particle. Thus, any microsphere in the microscope field can be used to calibrate blue, green and red fluorescent signals.

Our TetraSpeck™ fluorescent microspheres expand the multi-spectral strategy introduced with the MultiSpeck beads in two important ways. First, the TetraSpeck beads have been stained throughout with four different fluorescent dyes yielding beads that each display four well-separated excitation and emission peaks — 365/430 nm (blue), 505/515 nm (green), 560/580 nm (orange) and 660/680 nm (dark red). Second, these microspheres are available in five nominal sizes (actual bead diameters are indicated on product labels), spanning the range from sub-resolution to nearly cell-size particles: 0.1 μm, 0.2 μm, 0.5 μm, 1.0 μm and 4.0 μm.

Our FocalCheck™ fluorescent microspheres are specifically designed for examining the alignment, sensitivity and stability of confocal laser scanning microscopes. These 6 μm and 15 μm polystyrene beads have been treated by a proprietary method in which fluorescent dye is used to stain only the outermost portion of each microsphere. The resulting beads have a well-defined dye layer, which, when viewed in cross section in the confocal laser scanning microscope, appears as a fluorescent ring. The excitation/emission maxima exhibited by the different stains in the FocalCheck microspheres — blue (365/430 nm), green (505/515 nm), orange (560/580 nm) and dark red (660/680 nm) — are well matched to the laser sources and optical filters commonly used in confocal laser scanning microscopy. FocalCheck microspheres are currently available in several different configurations, including some exhibiting ring stains of two or three different fluorescent colors and others exhibiting a ring stain of one fluorescent color combined with a stain of a second fluorescent color throughout the bead.

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

<b>Cat #</b>	<b>Product Name</b>	<b>Unit Size</b>
P7220	PS-Speck™ Microscope Point Source Kit *blue, green, orange and deep red fluorescent beads* .....	1 kit

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## 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.

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### **Molecular Probes, Inc.**

29851 Willow Creek Road, Eugene, OR 97402  
Phone: (541) 465-8300 • Fax: (541) 335-0504

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### **Molecular Probes Europe BV**

Poortgebouw, Rijnsburgerweg 10  
2333 AA Leiden, The Netherlands  
Phone: +31-71-5233378 • Fax: +31-71-5233419

**Customer Service:** 9:00 to 16:30 (Central European Time)  
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[eurorder@probes.nl](mailto:eurorder@probes.nl)

### **Technical Assistance:** 9:00 to 16:30 (Central European Time)

Phone: +31-71-5233431 • Fax: +31-71-5241883  
[eurotech@probes.nl](mailto:eurotech@probes.nl)

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