

## TetraSpeck™ Fluorescent Microsphere Standards

**Table 1.** Contents and storage information.

Material	Amount	Concentration	Storage	Stability
Individual TetraSpeck™ Microsphere products	0.5 mL	Suspensions in deionized water, with 2 mM sodium azide.*	<ul style="list-style-type: none"> <li>• 2–6°C*</li> <li>• Protect from light</li> <li>• DO NOT FREEZE</li> </ul>	When stored properly, we guarantee the utility of TetraSpeck™ standards for a minimum of 12 months.
TetraSpeck™ Fluorescent Microspheres Sampler Kit (T7284): <ul style="list-style-type: none"> <li>• 0.1 µm TetraSpeck™ microspheres, (Component A)</li> <li>• 0.5 µm TetraSpeck™ microspheres, (Component B)</li> <li>• 4.0 µm TetraSpeck™ microspheres, (Component C)</li> </ul>	100 µL	Suspensions in deionized water, with 2 mM sodium azide.*	<ul style="list-style-type: none"> <li>• 2–6°C*</li> <li>• Protect from light</li> <li>• DO NOT FREEZE</li> </ul>	
TetraSpeck™ Fluorescent Microspheres Size Kit (T14792): <ul style="list-style-type: none"> <li>• Position 1: mounted sample of 4.0 µm TetraSpeck™ microspheres</li> <li>• Position 2: mounted sample of 1.0 µm TetraSpeck™ microspheres</li> <li>• Position 3: mounted sample of 0.5 µm TetraSpeck™ microspheres</li> <li>• Position 4: mounted sample of 0.2 µm TetraSpeck™ microspheres</li> <li>• Position 5: mounted sample of 0.1 µm TetraSpeck™ microspheres</li> <li>• Position 6: mounted mixture of all five sizes of TetraSpeck™ microspheres</li> </ul>	NA	NA	<ul style="list-style-type: none"> <li>• 2–6°C†</li> <li>• Protect from light</li> <li>• DO NOT FREEZE</li> </ul>	

\*See Bead Densities below for exact bead densities of the TetraSpeck™ products. †TetraSpeck™ slides can be stored at room temperature, protected from light

## Introduction

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Daily calibration and instrumentation adjustment are required for high-precision imaging of fluorescent probes, particularly in multicolor applications such as confocal laser scanning microscopy that involve multiple exposures, repetitive scans, or three-dimensional sectioning. Invitrogen's TetraSpeck™ fluorescent microspheres can greatly facilitate the calibration of conventional fluorescence microscopes, confocal laser scanning microscopes, and associated image-processing equipment for both scientific and commercial imaging, especially for multi-color applications. These uniform, multiply stained microspheres are particularly useful for verifying the ability of instrumentation to co-localize and resolve objects emitting different wavelengths of light in the same optical plane.

TetraSpeck™ microspheres are stained throughout with four different fluorescent dyes, yielding beads that each display four well-separated excitation/emission peaks—365/430 nm (blue), 505/515 nm (green), 560/580 nm (orange), and 660/680 nm (dark red). These microspheres are available in five diameters, spanning the range from subresolution to nearly cell-size particles:

- T7279, 0.1 μm diameter
- T7280, 0.2 μm diameter
- T7281, 0.5 μm diameter
- T7282, 1.0 μm diameter
- T7283, 4.0 μm diameter

The 0.1 μm and 0.2 μm TetraSpeck™ beads are ideal as subresolution fluorescent sources for calibrating instrument optics, especially in three-dimensional applications.

For convenience, we offer the TetraSpeck™ Fluorescent Microspheres Sampler Kit (T7284), containing separate samples of our 0.1 μm, 0.5 μm, and 4.0 μm TetraSpeck™ beads. We also offer the TetraSpeck™ Fluorescent Microspheres Size Kit (T14792), which includes a sample of each size of TetraSpeck™ microspheres, plus a mixture of each size, mounted on one slide.

### Bead Densities

The bead densities vary depending upon the bead diameters:  $\sim 1.8 \times 10^{11}$  particles/mL for the 0.1 μm beads;  $\sim 2.3 \times 10^{10}$  particles/mL for the 0.2 μm beads;  $\sim 1.5 \times 10^9$  particles/mL for the 0.5 μm beads;  $\sim 9.1 \times 10^8$  particles/mL for the 1.0 μm beads; and  $\sim 1.4 \times 10^7$  particles/mL for the 4.0 μm beads. The 0.1 μm, 0.2 μm, 0.5 μm, 1.0 μm, and 4.0 μm sizes are nominal values; actual diameters are indicated on the labels.



**Figure 1.** The TetraSpeck™ Fluorescent Microspheres Size Kit includes a sample of each size of TetraSpeck™ microspheres mounted on one slide. Position 1 contains 4.0 μm microspheres; position 2 contains 1.0 μm microspheres; position 3 contains 0.5 μm microspheres; position 4 contains 0.2 μm microspheres; position 5 contains 0.1 μm microspheres; and position 6 contains a mixture of all of the sizes.

## Guidelines for Use

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Invitrogen's TetraSpeck™ microspheres serve as reference standards for the calibration of conventional or confocal laser scanning microscopes and associated image-processing systems. Experimental protocols depend somewhat on the instrument and software used; please refer to the materials applicable to your particular instrument. The following serves as a guideline for mounting TetraSpeck™ microspheres on microscope slides. Note that the TetraSpeck™ Fluorescent Microspheres Size Kit (T14792) contains a slide of pre-mounted TetraSpeck™ microspheres.

1. Use clean glass microscope slides, i.e. oil and dust-free. Special cleaning is usually not required.
2. If desired, the beads in suspension can be diluted with distilled water before use. Before sampling, be sure that the beads are uniformly suspended by mixing on a vortex mixer or by sonicating.
3. Apply 5  $\mu\text{L}$  of the TetraSpeck™ bead suspension to the surface of a slide and spread with the pipette tip. Wait for the droplet to dry and then apply  $\sim 5 \mu\text{L}$  of glycerol or other mounting medium, such as water or immersion oil over the dry sample of beads. Some immersion oils may gradually extract dye from the microspheres, resulting in diminished bead fluorescence and increased background fluorescence. Consequently, the durability of slides prepared using oil may be limited.
4. Cover the sample with a coverslip. Seal the coverslip with nail polish, quick-drying glue, or melted paraffin.

## General References

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- Pawley, James, ed. 1995. *Handbook of Biological Confocal Microscopy*. 2nd edition. Plenum Press, New York.
- Conn, P. Michael, ed. 1990. *Quantitative and Qualitative Microscopy (Methods in Neurosciences, Vol. 3)*. Academic Press, New York.
- James, J. and Tanke, H. 1991. *Biomedical Microscopy*. J. Klubwer Academic Publishers, Dordrecht.

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

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Cat #	Product Name	Unit Size
T14792	TetraSpeck™ Fluorescent Microspheres Size Kit *mounted on slide* .....	1 kit
T7279	TetraSpeck™ microspheres, 0.1 $\mu\text{m}$ , fluorescent blue/green/orange/dark red .....	0.5 mL
T7280	TetraSpeck™ microspheres, 0.2 $\mu\text{m}$ , fluorescent blue/green/orange/dark red .....	0.5 mL
T7281	TetraSpeck™ microspheres, 0.5 $\mu\text{m}$ , fluorescent blue/green/orange/dark red .....	0.5 mL
T7282	TetraSpeck™ microspheres, 1.0 $\mu\text{m}$ , fluorescent blue/green/orange/dark red .....	0.5 mL
T7283	TetraSpeck™ microspheres, 4.0 $\mu\text{m}$ , fluorescent blue/green/orange/dark red .....	0.5 mL
T7284	TetraSpeck™ Fluorescent Microspheres Sampler Kit .....	1 kit

## Contact Information

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