

FocalCheck™ Fluorescent Microsphere Standards

Quick Facts

Storage upon receipt:

- 2–6°C, for microsphere suspensions
- Room temperature, for microsphere slides
- Do not freeze
- Protect from light

Introduction

Molecular Probes' Focal Check™ fluorescent microspheres are specifically designed for examining the alignment, sensitivity and stability of confocal laser scanning microscopes.¹ They are particularly useful for confirming the optical sectioning thickness ("Z-resolution") in three-dimensional imaging applications. These polystyrene beads, available in either 15 μm or 6 μm diameters, have been prepared 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 of varying dimensions depending on the focal plane (Figure 1). We refer to this proprietary staining procedure as ring staining, in order to differentiate it from routine staining throughout the bead.

FocalCheck products are currently available in several different multicolored configurations of fluorescent ring stains and/or contrasting fluorescent stain throughout the bead (Table 1). The excitation/emission maxima exhibited by the different stains in these 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 microscopes. Moreover, because the dyes are localized *within* the bead and therefore protected from environmental factors, the FocalCheck microspheres are brighter and more photostable than conventional surface-stained beads.

The sharp ring stains exhibited by the FocalCheck microspheres produce a striking visual representation of instrument misalignment or other aberrations, making them ideal as reference standards for confocal laser scanning microscopy. Correct image registration is indicated when the multiple ring images of the ring-stained FocalCheck beads (or the ring and disk images of the combination ring-stained and stained-throughout FocalCheck beads) are perfectly coincident in all dimensions (Figure 1). Furthermore, because the FocalCheck beads are available in a number of multicolor options, they are especially useful in testing and aligning confocal laser scanning microscopes that have multiple laser lines and detection channels.

Table 1. FocalCheck fluorescent microsphere suspensions and kits.

Cat #	Bead Size (μm)	Ring Stain *	General Stain * (Throughout)
F-7234	15	blue, orange	none
F-7240	15	green, dark red	none
F-7235 †	15	green, orange, dark red	none
F-14806 ‡	6	green, orange, dark red	none
F-7237 †	15	green	blue
F-14808 ‡	6	green	blue
F-7238	15	green	dark red
F-7236	15	orange	blue
F-7239 †	15	dark red	green
F-14807 ‡	6	dark red	green
F-36905 §	6	green-1 (500/512)	green-2 (512/525)
F-36906 §	6	orange-1 (532/552)	orange-2 (545/565)
F-36907 §	6	red-1 (580/610)	red-2 (569/574)
F-36908 §	6	far-red-1 (665/695)	far-red-2 (640/674)

* Ring and general stains are identified by fluorescence emission color.
 † Also available mounted on slides in Kit F-24634.
 ‡ Also available mounted on slides in Kit F-24633.
 § Only available mounted on slides as a kit. The fluorescent stains are spectrally similar, but can be resolved by the technique of spectral unmixing. The numbers in parentheses refer to the absorption and emission maxima for the respective dyes, in nm.

Special FocalCheck microspheres are available for testing spectral separation. These microspheres are stained with two different fluorescent dyes, which appear similar in color by eye. However, when linear-unmixing data-processing algorithms are applied, the dyes can be spectrally resolved — one appearing only within the outer ring and the other throughout. These 6 μm, dual-stained microspheres are provided mounted on slides in the FocalCheck DoubleGreen, DoubleOrange, DoubleRed and DoubleFarRed Fluorescent Microspheres Kits (F-36905, F-36906, F-36907, F-36908). In addition, the kits contain mounted control microspheres that have been stained throughout with each of the two dyes separately.

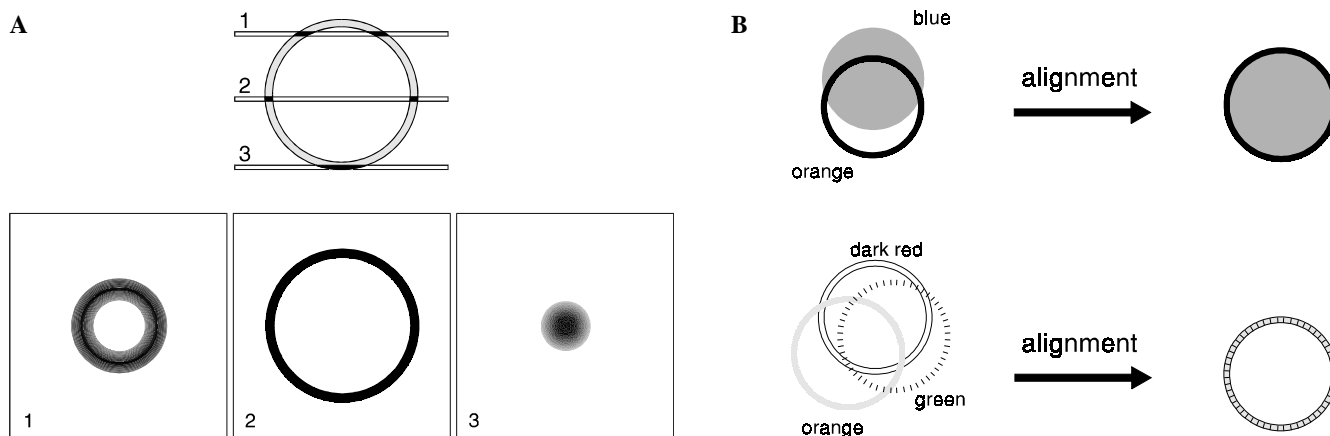


Figure 1. Confocal laser-scanning microscope optical cross sectioning and alignment with FocalCheck microspheres. A) The diameter of the fluorescent ring (or disk) seen is dependent on the depth of the optical focal plane. B) In the confocal laser-scanning microscope, separate light paths exist for UV and visible wavelengths. Also, fluorescence emitted is detected by different photomultiplier detectors. Proper optical alignment may be obtained with either of two types of FocalCheck microspheres. For example, the orange ring stain/blue throughout microspheres allow UV/visible wavelength alignment in three dimensions upon aligning the orange ring with the blue disk. Focal alignment is also possible simultaneously in three colors by aligning the green, orange and dark red rings of the FocalCheck microspheres containing fluorescent green/orange/dark red ring stains.

Materials

FocalCheck Microsphere Suspensions

FocalCheck microsphere suspensions are provided in deionized water containing 0.02% Tween® 20 and 2 mM sodium azide, each with a unit size of 0.5 mL. The suspension densities are $\sim 5 \times 10^5$ beads/mL (0.1% solids) for the 15 μm beads and $\sim 8 \times 10^6$ beads/mL (0.1% solids) for the 6 μm beads. The nominal bead diameters are 15 μm or 6 μm ; actual diameters are indicated on the product labels. The bead diameters have very small coefficients of variation, typically <3%.

Upon receipt, FocalCheck microsphere suspensions should be stored at 2–6°C, protected from light. DO NOT FREEZE. When stored properly, we guarantee the utility of FocalCheck standards for a minimum of six months from date of purchase.

FocalCheck Microspheres Mounted on Slides

FocalCheck microspheres are also available mounted on slides. The FocalCheck Fluorescent Microspheres Kit, 6 μm (F-24633) features one slide each of product numbers F-14806, F-14807 and F-14808 (see Table 1); the FocalCheck Fluorescent Microspheres Kit, 15 μm (F-24634) features one slide each of product numbers F-7235, F-7237 and F-7239.

The FocalCheck Microspheres mounted on slides may be stored at room temperature, protected from light. DO NOT FREEZE.

FocalCheck Microspheres for Testing Spectral Separation, Mounted on Slides

The FocalCheck DoubleGreen, DoubleOrange, DoubleRed and DoubleFarRed Microspheres Kits each include three slides. One slide contains microspheres having a ring stain of one fluorescent dye and a stain throughout of a similar fluorescent color. The dyes are spectrally similar, but sufficiently different to be resolved by the technique of linear unmixing in instruments such as the Zeiss LSM META™ fluorescence microscope. Two additional slides, to serve as controls, contain microspheres stained uniformly with individual dyes. Table 1 contains absorption and emission maxima for the dyes contained within these microspheres.

Sample Preparation

Molecular Probes' FocalCheck microspheres serve as reference standards for the calibration of confocal-microscopes. 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 FocalCheck microspheres on microsphere slides.

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, make sure that the beads are uniformly suspended by mixing on a vortex mixer or sonicating.
3. Apply 5 μL of the FocalCheck bead suspension to the surface of a slide and spread with the pipette tip. Wait for the droplet to dry and then apply 5 μL 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

- 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.

References

1. Appl Immunohistochem Mol Morphol 7, 156 (1999).

Product List

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

Cat #	Product Name	Unit Size
F-36908	FocalCheck™ DoubleFarRed Fluorescent Microspheres Kit, 6 µm *mounted on slides*	1 kit
F-36905	FocalCheck™ DoubleGreen Fluorescent Microspheres Kit, 6 µm *mounted on slides*	1 kit
F-36906	FocalCheck™ DoubleOrange Fluorescent Microspheres Kit, 6 µm *mounted on slides*	1 kit
F-36907	FocalCheck™ DoubleRed Fluorescent Microspheres Kit, 6 µm *mounted on slides*	1 kit
F-24633	FocalCheck™ Fluorescent Microspheres Kit, 6 µm *mounted on slides*	1 kit
F-24634	FocalCheck™ Fluorescent Microspheres Kit, 15 µm *mounted on slides*	1 kit
F-14807	FocalCheck™ microspheres, 6.0 µm, fluorescent dark-red ring stain/green throughout	0.5 mL
F-14806	FocalCheck™ microspheres, 6.0 µm, fluorescent green/orange/dark-red ring stains	0.5 mL
F-14808	FocalCheck™ microspheres, 6.0 µm, fluorescent green ring stain/blue throughout	0.5 mL
F-7234	FocalCheck™ microspheres, 15 µm, fluorescent blue/orange ring stains	0.5 mL
F-7239	FocalCheck™ microspheres, 15 µm, fluorescent dark-red ring stain/green throughout	0.5 mL
F-7240	FocalCheck™ microspheres, 15 µm, fluorescent green/dark-red ring stains	0.5 mL
F-7235	FocalCheck™ microspheres, 15 µm, fluorescent green/orange/dark-red ring stains	0.5 mL
F-7237	FocalCheck™ microspheres, 15 µm, fluorescent green ring stain/blue throughout	0.5 mL
F-7238	FocalCheck™ microspheres, 15 µm, fluorescent green ring stain/dark red throughout	0.5 mL
F-7236	FocalCheck™ microspheres, 15 µm, fluorescent orange ring stain/blue throughout	0.5 mL

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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