

## FocalCheck™ Thin-Ring Fluorescent Microspheres Kit (F-14791)

### Quick Facts

#### Storage upon receipt:

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

**Note:** Sonicate before use

- Component A: Microspheres with green and red ring stains
- Component B: Microspheres with a green ring stain and a dark red stain throughout
- Component C: Microspheres with a red ring stain and a blue stain throughout

All of the suspensions are in deionized water containing 2 mM sodium azide, at a density of  $\sim 3 \times 10^8$  beads/mL (0.02% solids).

### Storage

Upon receipt, FocalCheck Thin-Ring microspheres should be stored at 4°C, protected from light. **DO NOT FREEZE.** When stored properly, we guarantee the utility of FocalCheck standards for at least six months from date of purchase.

### Introduction

Molecular Probes' **FocalCheck™ Thin-Ring Fluorescent Microspheres Kit** (F-14791) is designed to help you examine the alignment, sensitivity and stability of your confocal laser scanning microscope. The kit provides three suspensions of 1  $\mu\text{m}$ -diameter, uniquely stained microspheres. For each suspension, the beads have been stained on the surface with one fluorescent color and with a second contrasting fluorescent color, either on the surface again or throughout. Thus, when viewed in cross section with a confocal laser scanning microscope, the beads exhibit a sharp ring pattern coupled with either a second ring pattern or a bright disk pattern of a different color.

The sharp ring stains exhibited by the FocalCheck Thin-Ring 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 ring images of the doubly ring-stained beads (or the ring and disk images of the combination ring-stained and stained-throughout beads) are perfectly coincident in all dimensions.

We have carefully selected the fluorescent microsphere stains so that the excitation/emission maxima of the dyes match the major emission lines of the Kr-Ar and UV argon-ion lasers found on the most commonly used confocal microscopes. The excitation/emission maxima exhibited by the different stains in these microspheres — blue (365/430 nm), green (495/515 nm), red (575/600 nm) and dark red (660/680 nm) — are well matched to the laser sources and optical filters commonly used in confocal laser scanning microscopes.

### Contents and Storage

#### Contents

The FocalCheck Thin-Ring Fluorescent Microspheres Kit contains three 200  $\mu\text{L}$  suspensions of 1  $\mu\text{m}$ -diameter microspheres:

### Sample Preparation

Molecular Probes' FocalCheck Thin-Ring microspheres serve as reference standards for the calibration of confocal laser scanning 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.

**1.1** Use clean glass microscope slides, i.e. oil and dust-free. Special cleaning is usually not required.

**1.2** 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.

**1.3** Apply 5  $\mu\text{L}$  of the FocalCheck Thin-Ring 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.

**1.4** Cover the sample with a coverslip. Seal the coverslip with nail polish, quick-drying glue or melted paraffin.

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## References

1. Abrams, S., 1998. *Biophotonics International*, (May/June). Laurin Publications; 2. Conn, P. Michael, ed., 1990. *Quantitative and Qualitative Microscopy (Methods in Neurosciences, Vol. 3)*. Academic Press, New York; 3. James, J. and Tanke, H., 1991. *Biomedical Microscopy*. J. Klubwer Academic Publishers, Dordrecht; 4. Pawley, James, ed., 1995. *Handbook of Biological Confocal Microscopy*, 2nd edition. Plenum Press, New York.

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## Product Summary

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

Cat #	Product Name	Unit Size
F-14791	FocalCheck™ Thin-Ring Fluorescent Microspheres Kit *three suspensions* .....	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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