

InSpeck™ Microscope Image Intensity Calibration Kits

Quick Facts

Storage upon receipt:

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

Ex/Em: See Table 1

Introduction

InSpeck™ Microscope Image Intensity Calibration Kits provide fluorescence microscopists with intensity references for generating calibration curves and evaluating sample brightness. The kits are offered in a choice of five fluorescent colors, allowing for calibrations over a wide range of excitation and emission wavelengths (Table 1). Each kit includes separate vials of 2.5 or 6.0 μm-diameter InSpeck fluorescent microspheres with fluorescence intensities ranging from very low-intensity fluorescence to the brightest signal expected in most microscopy applications. The kits also contain a vial of nonfluorescent microspheres for use as a control. These aqueous suspensions of microspheres may be applied directly to the sample for calibrating fluorescence intensities or mounted separately in an adjacent well or on a slide.

Table 1. InSpeck Microscope Image Intensity Calibration Kits.

Cat #	InSpeck Kit	Bead Diam.*	Ex/Em †
I-7221	InSpeck Blue	2.5	350/440
I-7219	InSpeck Green	2.5	505/515
I-14785	InSpeck Green	6.0	
I-7223	InSpeck Orange	2.5	540/560
I-14786	InSpeck Orange	6.0	
I-7224	InSpeck Red	2.5	580/605
I-14787	InSpeck Red	6.0	
I-7225	InSpeck Deep Red	2.5	633/660

* Approximate microsphere diameter, in μm. † Approximate excitation and emission maxima, in nm, for the stained microspheres.

Materials

Contents

Each InSpeck Microscope Image Intensity Calibration Kit includes seven vials of either 2.5 or 6.0 μm-diameter polystyrene microspheres, plus a vial of mounting medium:

- **Nonfluorescent microspheres** (Component A)
- **0.3% Relative intensity fluorescent microspheres** (Component B)
- **1% Relative intensity fluorescent microspheres** (Component C)
- **3% Relative intensity fluorescent microspheres** (Component D)
- **10% Relative intensity fluorescent microspheres** (Component E)
- **30% Relative intensity fluorescent microspheres** (Component F)
- **100% Relative intensity fluorescent microspheres** (Component G)
- **Mounting medium** (Component H), 5 mL

The microspheres are provided as 1 mL suspensions in water containing Tween® 20 and 2 mM sodium azide. The microspheres are at a density of 0.05% solids ($\sim 6 \times 10^7$ beads/mL for the 2.5 μm microspheres or $\sim 4 \times 10^6$ beads/mL for the 6.0 μm microspheres). Each 1 mL suspension provides enough material to mount about 100 slides. Note that the mounting medium provided does not harden or gel.

The fluorescence intensity of the particles is defined with reference to that of the microspheres with the brightest fluorescence. The fluorescence intensity of the microspheres in each suspension is very homogeneous (see Figure 1). The mean fluorescence intensities for the samples in the InSpeck Blue Kit are determined using a Becton Dickinson FACS Vantage™ flow cytometry and 365 nm excitation. The mean fluorescence intensities for the samples in the InSpeck Green, Orange, Red and Deep Red Kits are determined using a Becton Dickinson FACScan™ flow cytometer and 488 nm excitation. The actual relative fluorescence intensities of Components B, C, D, E and F may vary somewhat from the values listed above, depending on the kit and the production lot; the mean value is specified on each vial. Note that the InSpeck microspheres are designed primarily for use in conventional fluorescence microscopy. If used in laser-scanning confocal microscopy, the individual dim microspheres, viewed in cross section, may appear non-uniformly stained — the staining may be more intense in the periphery than in the center.

Storage and Handling

Upon receipt, the kit components should be stored at 4°C, protected from light. DO NOT FREEZE. The microsphere suspensions are provided at a concentration appropriate for most applications. Each vial should be agitated in a vortex mixer or sonicator bath immediately before sampling to insure a homogeneous suspension. If desired, the particle concentration can be increased or the solution exchanged by centrifuging the suspension at about 12,000 × g for 2 minutes in a microcentrifuge. The particle concentration can be reduced by diluting with water.

Applications

Internal Fluorescence Microscopy Standards

InSpeck microspheres can be incorporated into experimental samples to serve as internal intensity standards. Add a small drop (<10 µL for a standard coverslip-size sample) of one of the InSpeck suspensions directly to the sample and visualize with a fluorescence microscope equipped with an appropriate filter set.

External Fluorescence Microscopy Standards

InSpeck microspheres can be mounted on a microscope slide and used as an external intensity standard. Add a small drop (<10 µL for a standard coverslip-size sample) to a microscope slide or coverslip and air dry; protect from dust during drying. To make it easy to identify the position of the microspheres once the drop dries, we recommend that you use microscope slides that have etched rings or circles drawn with a marking pen on the underside. Alternatively, you can dry the droplet on the coverslip instead of on the slide itself. In either method, when the sample is completely dry, add a small drop of mounting medium to cover the spot, place the coverslip on the slide and seal. The mounting medium will remain liquid; thus, the sample distribution may not be permanent. Visualize the mounted stan-

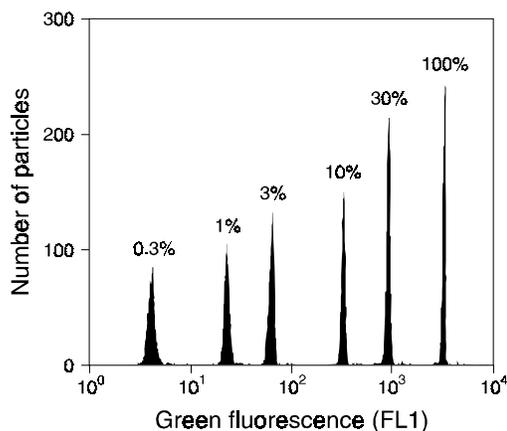


Figure 1. Fluorescence intensity histogram of the six fluorescent microsphere samples supplied in our InSpeck Green (505/515) Microscope Image Intensity Calibration Kit, 2.5µm. Fluorescence measurements were performed on a Becton Dickinson FACScan flow cytometer using excitation at 488 nm. The nominal relative fluorescence intensities are indicated; the actual measured values for this lot are 0.2%, 0.8%, 2.3%, 10.6%, 30.0%, and 100%, respectively.

dards with a fluorescence microscope equipped with an appropriate filter set.

Multi-Intensity Applications

When preparing intensity calibration curves, it may be desirable to mix the InSpeck intensity standards 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.

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

Cat #	Product Name	Unit Size
I-7221	InSpeck™ Blue (350/440) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit
I-7225	InSpeck™ Deep Red (633/660) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit
I-7219	InSpeck™ Green (505/515) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit
I-14785	InSpeck™ Green (505/515) Microscope Image Intensity Calibration Kit, 6.0 µm	1 kit
I-7223	InSpeck™ Orange (540/560) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit
I-14786	InSpeck™ Orange (540/560) Microscope Image Intensity Calibration Kit, 6.0 µm	1 kit
I-7224	InSpeck™ Red (580/605) Microscope Image Intensity Calibration Kit, 2.5 µm	1 kit
I-14787	InSpeck™ Red (580/605) Microscope Image Intensity Calibration Kit, 6.0 µm	1 kit

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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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