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MultiSpeck™ Multispectral Fluorescence Microscopy Standards Kit (M-7901)

Quick Facts

Storage upon receipt:
- 4°C
- Do not freeze
- Protect from light

Introduction

The MultiSpeck™ Multispectral Fluorescence Microscopy Standards Kit provides researchers with an external reference for comparing images collected with different optics, on different instruments and in different laboratories, as well as for monitoring routine day-to-day variations in instrumental performance.

The kit includes two suspensions of 4.0 μm–diameter microspheres. The first suspension, the MultiSpeck suspension, is comprised of multispectral fluorescent microspheres that exhibit three relatively distinct excitation and emission bands — red, green and blue — all in the same particle. When excited with ultraviolet light, each sphere emits blue fluorescence, whereas when fluorescein or rhodamine/Texas Red® excitation filters are used, the spheres fluoresce at wavelengths similar to fluorescein or rhodamine/Texas Red emissions, respectively. Furthermore, because a single multispectral microsphere will appear different colors depending on the filters used for observation, these microspheres can be used for image registration in two and three dimensions, thus allowing the researcher to accurately determine the spatial relationships of multiple labels in a multiparameter experiment. The second suspension, the RGB Mix suspension, is comprised of a mixture of "single-band" microspheres that exhibit the same three excitation/emission bands — red, green and blue (RGB) — as the multispectral microspheres, but in separate particles.

Both suspensions in the kit are provided at ready-to-use densities and can be mounted on slides or incorporated into an experimental sample. The kit also includes nonhardening mounting medium and instructions for mounting the microspheres on slides.

Contents and Storage

Contents

The MultiSpeck Multispectral Fluorescence Microscopy Standards Kit contains two aqueous suspensions of 4.0 μm–diameter polystyrene microspheres, plus mounting medium:

- **MultiSpeck multispectral microspheres** (Component A), 0.5 mL suspension
- **RGB Mix microspheres** (Component B), 0.5 mL suspension
- **Mounting medium** (Component C), 5 mL

Each microsphere suspension provides enough material to prepare about 50 slides. Note that the mounting medium provided does not harden or gel (see *External Fluorescence Microscopy Standards*).

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 of ~3×10^6 beads/mL (0.01% solids). 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.

Spectral Properties

The MultiSpeck multispectral microspheres (Component A) are formulated to exhibit excitation and emission profiles with peak positions and bandwidths that maximize the similarity between a wide variety of dyes and dye complexes, while minimizing the spectral overlap between the separate excitation and emission components of the multispectral bands. Thus, the excitation and emission profiles are not continuous but somewhat discrete and separable.

In contrast to the multispectral microspheres in which the three excitation/emission spectra are integrated in a single particle, the microspheres in the RGB Mix suspension (Component B) contain the three excitation/emission spectra in a mixture of three individual microsphere populations: blue fluorescent

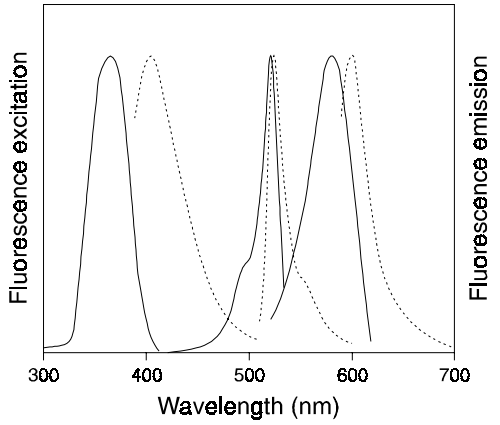

Figure 1. *Excitation and emission spectra for microspheres separately stained with the dyes used for MultiSpeck microspheres. The excitation (solid lines) and emission spectra (dotted lines) for fluorescent blue (left pair), green (center pair) and red microspheres (right pair) are normalized and plotted in a common graph.*

microspheres, green fluorescent microspheres and red fluorescent microspheres. When analyzed separately, the blue fluorescent microspheres exhibit excitation/emission maxima of ~365/405 nm; the green fluorescent microspheres, ~520/525 nm; and the red fluorescent microspheres ~580/600 nm (Figure 1). Note that the spectral properties of the microspheres in the MultiSpeck Multispectral Fluorescence Microscopy Standards Kit have recently been recalibrated and may differ slightly from that of previous lots.

Sample Preparation

Internal Fluorescence Microscopy Standards

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

External Fluorescence Microscopy Standards

MultiSpeck multispectral and RGB Mix microspheres can be mounted on a microscope slide and used as an external fluorescent standard. Add a small drop (<10 µL for a standard coverslip-size sample) of one of the suspensions 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 the appropriate filter set.

Applications

Spectral Responses of Different Filters and Lenses

The MultiSpeck multispectral microspheres can be observed with a large number of optical filters because they contain a proprietary stain mixture that mimics the spectral properties of a wide variety of dyes and dye complexes. They will fluoresce different colors, depending on the filter used.

Image Registration

When using multiple filters with the MultiSpeck multispectral microspheres, you may see image registration differences; i.e., microspheres may appear to change position as a function of changing filter sets. This information can be used to compensate for differences in filter alignments and to identify the coincidence of multiple fluorescent labels independent of instrumental artifacts. Using MultiSpeck microspheres, researchers can accurately determine the spatial relationships of multiple labels in a multiparameter experiment within the optical resolution limit.

Multiband Filters

Multiband filters (more than one excitation and emission spectrum per filter) may pass a combination of colors emitted by the multispectral microspheres in the MultiSpeck suspension. Thus, the multispectral microspheres will not look pure blue, green or red, but will instead appear a unique color that results from the multiple emissions passed by the filter.

The single-band red, green and blue microsphere mixture in the RGB Mix suspension can be used to examine the relative excitation and emission efficiencies of multiband filter sets. The microspheres that have spectra corresponding to the bands in the filter set will exhibit either pure blue, green or red emission and the different colors will appear in different positions in the microscope field.

Product List

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

Cat #	Product Name	Unit Size
M-7901	MultiSpeck™ Multispectral Fluorescence Microscopy Standards Kit *in suspension*	1 kit

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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