

Color-Rich™ Fluoro-Max™ Dyed Microparticles

March 2008

Introduction

Thermo Scientific Seradyn dyed and fluorescent microparticles are monodisperse particles prepared by unique and proprietary emulsion polymerization methods. Currently, the largest use of the Color-Rich microparticles is in membrane-based clinical diagnostic tests and research applications. Color-Rich microparticles are made by dyeing OptiBind® polystyrene and OptiLink® carboxylate-modified particles with proprietary dyes.

Color-Rich Dyed Microparticles

The Color-Rich microparticles incorporate dyes internally. The most important characteristic for any dyed microparticle is visibility in the application, whether it is a visual or fluorescent microscopy technique. Internal dyeing of the microparticles insures maximum color brilliance and color saturation, prevents dye leaching in aqueous media, and leaves the surface free for optional immunological applications. The Color-Rich microparticles are available in specific diameters and colors. Colors available include blue and red. These microparticles have been specifically designed and are most often used for membrane-based applications. Consult the catalog for specific products.

Fluoro-Max Fluorescent Microparticles

The Fluoro-Max fluorescent particles are made by dyeing OptiLink particles with europium chelate and are available in standard 0.1 μM, 0.2 μM, and 0.3 μM diameters. They are dyed internally to prevent dye leaching and to assure maximum surface immunoreactivity. These particles have been specifically designed for membrane or automated fluorometric-based applications. Fluoro-Max particle data:

	Excitation	Emission
Europium Chelate	333 nm	613 nm

With an extremely broad Stokes shift, the europium chelate particles help prevent nonspecific fluorescence interference. The Fluoro-Max particles may be used in a variety of applications such as clinical diagnostics, immuno/histological studies and flow cytometry.

Applications

For many types of immunological assays, the dyed particles can enhance the readability of the assay or do away with problems associated with enzyme-based assays.

In addition to diagnostic assays and molecular biology applications, there are a variety of other applications where these dyed and fluorescent particles may be used:

- Membrane pore-size determinations
- Flow or fluid mechanism studies
- Immuno/histology studies
- Flow imaging tracers
- Biological transport studies
- Filtration media analysis
- Particle size correlation
- Phagocytosis research
- Cell surface markers

Fluoro-Max Technical Data

Fluorescence Spectral Characteristics Europium Chelate Particles

Excitation: 333 nm, 50% bandwidth = 65 nm, 90% bandwidth = 100 nm
Emission: 613 nm, 50% bandwidth = 14 nm, 90% bandwidth = 25 nm
Satellite fluorescence ratio, 613nm/591nm = 12

Abbreviations: NTA, naphyltrifluorobutanedione; Eu, europium; B-PE, B-phycoerythrin; UV, ultraviolet; quantum yield = light emitted/light absorbed.

Europium chelate particles are composed of carboxylate-modified polystyrene microparticles imbibed with Europium chelate. When excited with UV light at its maximal absorbance wavelength of 333 nm, europium chelate particles emit long-lived fluorescence at 613 nm, with a lifetime of approximately 0.5 milliseconds, which is about 10,000 to 100,000 times longer than the lifetimes of most fluorophores. This extremely long lifetime allows the application of europium chelate particles to time-resolved fluorescence. This also can reduce the detection limits of fluorescence assays many orders of magnitude by elimination of background interference from relatively short-lived matrix fluorescence.

Although the excitation (absorbance) spectrum of europium chelate is broad, the emission at 613 nm is very narrow and is accompanied by a much smaller satellite peak at 591 nm due to a magnetic dipole transition. The properties of the europium chelate particles are nearly identical to optimized soluble Europium chelate except that the fluorescence quantum yield is enhanced about 10% when imbibed into the particle. The chelate in solution has an absorptivity of 53,000 liter/mol-cm, with a quantum yield of approximately 0.2, and the concentration of chelate inside the particle corresponds to a particle (100% solids) absorbance of 5000.

The europium chelate particle has 100% fluorescence efficiency relative to

soluble chelate, in contrast to most fluorophores which strongly quench each other at high substitution levels. For a typical 100 nm size europium particle, the fluorescence yield is equivalent to about 3,000 molecules of fluorescein. By comparison, phycobiliprotein B-PE (perhaps the most fluorescent substance known) has a fluorescence yield equivalent to about 30 fluorescein molecules. Since a 100 nm particle is about 10 times the diameter of phycobiliprotein B-PE and a thousand times greater in volume/mass, Thermo Scientific Seradyn europium chelate particles are 100 times more fluorescent than B-PE on a molar basis, but only 10% as fluorescent on a volume/mass basis. In comparison to Molecular Probes fluorescent particle T-8871 (Ex/Em 495/512), Thermo Scientific Seradyn europium chelate particles are one-quarter as fluorescent on a mass basis (% solids), but nearly three times as fluorescent as Molecular Probes' fluorescent long-wavelength particle T-8878 (Ex/Em 580/690) on a molar basis.

**Color-Rich
Technical
Data**

Our technical staff performed IgG adsorption and covalent coupling studies with dyed and non-dyed stock microparticles. “The Microparticle-Bound Protein Assay” Technical Supplement details the process used to quantify the amount of protein binding. Results were as shown below (reported in µg IgG/mg microparticles).

Protein Binding to Dyed Particles

Our dyeing process causes little or no inhibition of adsorption or covalent coupling of IgG to our dyed microparticles. Our studies show no leaching of the dye during wash steps. There was no visible staining of the supernatants in these experiments.

Particle Type*	Total Bound IgG (µg/mg)	Covalently Bound IgG (µg/mg)
White 0.298 µM CM	92	91
Blue 0.298 µM CM	109	99
White 0.284 µM CM	108	105
Blue 0.284 µM CM	131	129
White 0.295 µM PS	77	---
Blue 0.295 µM PS	76	---

* CM = Carboxylate Modified; PS = Polystyrene

Please see the “Recommended Adsorption and Coupling Procedures” under Particle Technology Technical Notes Section of our Web site for full details on our validated coupling protocols and procedures.

Color-Rich

Particle Stability

Color-Rich Dyed Particle Stability

A four week stability study at 4 °C and 37 °C was performed on a 0.3 µM lot of dyed and non-dyed polystyrene microparticles. Each week the reaction samples from the IgG adsorbed particles were resuspended and tested for protein desorption using our Microparticle-Bound Protein Assay. The difference between the microparticle sample and the washed pellet was taken as the level of protein desorption. Stability test results were as follows:

	Time 0	Week 1		Week 2		Week 4	
		<u>4°</u>	<u>37°</u>	<u>4°</u>	<u>37°</u>	<u>4°</u>	<u>37°</u>
White Particle	77.4	70.6	78.3	68.0	68.8	72.2	67.4
White Pellet	78.8	70.7	77.9	63.6	61.5	68.8	56.3
Blue Particle	76.3	68.3	73.9	69.9	67.1	61.0	58.7
Blue Pellet	72.3	58.9	76.1	60.3	63.3	66.6	57.9

There is little or no desorption of protein at 37 °C or 4 °C from the dyed microparticles under the conditions of this experiment. Thorough resuspension of the reaction tubes is vitally important. A large portion of any inconsistent data can be attributed to this insufficient resuspension.

**Ordering
Color-Rich
and
Fluoro-Max
Microparticles**

Color-Rich Microparticles

(Nominal 2.5% solids concentration, 0.05% sodium azide)

Standard Products listed. Ask for additional custom material.

Color Rich-Polystyrene Catalog No.

0.3 μ M	PS Red	8120-0797
0.8 μ M	PS Red	8120-1797

Color Rich-Carboxylate Catalog No.

0.2 μ M	CM Blue	8310-0550
0.3 μ M	CM Blue	8310-0750
0.8 μ M	CM Blue	8310-1750

Fluoro-Max-Carboxylate Microparticles

(Nominal 1% solids concentration, 0.05% sodium azide)

Fluoro-Max-Carboxylate Catalog No.

0.1 μ M	CM Europium	9347-0350
0.2 μ M	CM Europium	9347-0550
0.3 μ M	CM Europium	9347-0750

Other Products

You are cordially invited to visit our facilities any time you are in the Indianapolis area. We are just minutes from the Indianapolis International Airport. Please inquire about our other exceptional microparticle products. We have a complete range of microparticles to suit your individual requirements.

Technical Help/Ordering Information

For technical help, call the Particle Technology Division or write, e-mail or fax to the address below. We'll be glad to help you in any way we can.

PRICE BASIS	PAYMENT TERMS
FOB Indianapolis, IN USA	Net 30 days to firms
Freight prepaid/added to invoice	of approved credit

ORDER ENTRY	CONTACT
Seradyn, Inc	
Subsidiary of Thermo Fisher Scientific Inc.	Tel. 800 428-4072
7998 Georgetown Road	317 610-3800
Indianapolis, IN 46268 USA	Fax: 317 610-3888

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www.thermo.com/particletechnology

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