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BioParticles® Fluorescent Particles and Opsonizing Reagents

***Escherichia coli* BioParticles**
***Staphylococcus aureus* BioParticles**
Zymosan A BioParticles

Quick Facts

Storage upon receipt:
- –20°C
- Desiccate
- Protect from light

Abs/Em: See Table 1

Introduction

Fluorescent Bacteria and Yeast Particles

Molecular Probes' BioParticles® product line consists of a series of fluorescently labeled, heat- or chemically killed bacteria and yeast in a variety of sizes, shapes and natural antigenicities. These fluorescent BioParticles products have been employed to study phagocytosis by fluorescence microscopy,[1] quantitative spectrofluorometry [2] and flow cytometry.[3-5] We offer *Escherichia coli* (K-12 strain), *Staphylococcus aureus* (Wood strain, without protein A) and zymosan (*Saccharomyces cerevisiae*) BioParticles conjugates covalently labeled with a variety of different fluorophores (Table 1); special care has been taken to remove free dye after conjugation. Unlike the fluorescence of fluorescein-labeled BioParticles conjugates, which is partially quenched in acidic environments, the fluorescence of the Alexa Fluor®, BODIPY® FL, tetramethylrhodamine and Texas Red® conjugates is uniformly intense over a broad pH range (pH 4–9). This property may be particularly useful in quantitating fluorescent bacteria and zymosan within acidic phagocytic vacuoles. Molecular Probes also offers nonfluorescent *S. aureus* and zymosan BioParticles. These nonfluorescent BioParticles products are useful both as controls or for custom-labeling with reactive dyes.

Fluorescent bacteria and yeast particles are proven tools for studying a variety of parameters influencing phagocytosis; for example, they have been used to:

- Detect the phagocytosis of yeast by murine peritoneal macrophage [6] and human neutrophils.[2]
- Determine the effects of different opsonization procedures on the efficiency of phagocytosis of pathogenic bacteria [7] and yeast.[2]
- Investigate the kinetics of phagocytosis degranulation and actin polymerization in stimulated leukocytes.[2]
- Measure phagocytosis and, in conjunction with dihydroethidium, oxidative bursts in leukocytes using flow cytometry.[8,9]
- Quantitate the effects of anti-inflammatory drugs on phagocytosis.[2]
- Show that *Dictyostelium discoideum* depleted of clathrin heavy chains are still able to undergo phagocytosis of fluorescent zymosans.[10]
- Study molecular defects in phagocytic function.[11]

The fluorescence of BioParticles conjugates that are bound to the surface but not internalized can be quenched by ethidium bromide,[1] trypan blue [12,13] or other quenchers. In addition to cellular applications, fluorescent BioParticles conjugates may be effective as flow cytometry calibration references when sorting bacteria and yeast mutants. These small particles may also be useful references for light scattering studies because their sizes and shapes differ in characteristic ways.

Table 1. *Molecular Probes' BioParticles products.*

Fluorophore (Abs/Em) *	Type of Particle and Catalog #		
	E. coli	*S. aureus*	Zymosan (*S. cerevisiae*)
Fluorescein (494/518)	E-2861	S-2851	Z-2841
Alexa Fluor 488 (495/519)	E-13231	S-23371	Z-23373
BODIPY FL (505/513)	E-2864	S-2854	Z-2844
Tetramethylrhodamine (555/580)	E-2862		
Alexa Fluor 594 (590/617)	E-23370	S-23372	Z-23374
Texas Red (595/615)	E-2863		Z-2843
Unlabeled		S-2859	Z-2849
Opsonizing Reagent	E-2870	S-2860	Z-2850

* Approximate absorption (Abs) and fluorescence emission (Em) maxima, in nm.

Opsonizing Reagents

Many researchers may want to use autologous serum to opsonize their fluorescent zymosan and bacterial particles; however, we also offer special opsonizing reagents for enhancing the uptake of each type of particle. These reagents are derived from

purified rabbit polyclonal IgG antibodies that are specific for the *E. coli*, *S. aureus* or zymosan particles.

Materials

BioParticles Fluorescent Bacteria and Yeast Particles

E. coli, *S. aureus* and zymosan A BioParticles products are provided as lyophilized powders. Except for the Alexa Fluor BioParticles conjugates, which are packaged in a unit size of 2 mg, the fluorescent BioParticles conjugates are packaged in unit sizes of 10 mg. The unlabeled BioParticles materials are packaged in unit sizes of 100 mg. There are approximately 3×10^8 *E. coli* or *S. aureus* particles per mg solid and approximately 2×10^7 zymosan A particles per mg solid.

Upon receipt, the BioParticles products should be stored frozen at -20°C, desiccated and, in the case of fluorescently labeled products, protected from light. The products stored under these conditions are stable for at least one year. Reconstitution and storage after reconstitution are described in *Reconstitution and Use of BioParticles Fluorescent Bacteria and Yeast*, below.

BioParticles Opsonizing Reagents

BioParticles opsonizing reagents are provided lyophilized. Each vial (one unit) is sufficient to opsonize 10 mg of the corresponding type of BioParticles conjugate. In addition to rabbit polyclonal IgG antibodies specific for *E. coli*, *S. aureus* or yeast particles, the opsonizing reagents contain high-quality, RIA-grade bovine serum albumin (500 µg per vial) to block nonspecific binding. Upon receipt, BioParticles opsonizing reagents should be stored desiccated at -20°C, where they will remain stable for at least one year. Reconstitution is described in *Reconstitution and Use of Opsonizing Reagents*, below.

Application

Reconstitution and Use of BioParticles Fluorescent Bacteria and Yeast

The fluorescently labeled and unlabeled BioParticles bacteria and yeast can be reconstituted in a glass tube or vial at 20 mg/mL in the buffer of your choice (e.g. tissue culture–grade PBS with or without 2 mM sodium azide). Cap the tube and gently swirl the particles into suspension. Then, vigorously vortex the particles (3 × 15 seconds at the highest setting). Reconstituted suspensions can be stored at 4°C for several weeks with the addition of sodium azide to a final concentration of 2 mM. Without the addition of sodium azide, reconstituted suspensions should be used within one day. Protect suspensions of fluorescent BioParticles conjugates from light.

Aggregation can occur during freeze drying; the presence of aggregates will affect particle counts. To obtain as homogenous a suspension as possible, sonicate the particles (we use 3 × 20 seconds; 45–50 kilohertz; 55–90 ultrasonic watts; Bransonic 1200 Ultracleaner, Branson Ultrasonics, Danbury, CT). Dilute a small volume of the particle suspension about 50–300-fold and inspect the suspension for its uniformity of dispersion. Sonicate until the particles are suspended homogeneously.

Fluorescently labeled and unlabeled BioParticles bacteria and yeast can be used with or without autologous serum, with your own opsonizing reagents or with specific (IgG) opsonizing reagents from Molecular Probes. After obtaining as homogenous a suspension as possible, opsonize and wash the BioParticles bacteria or yeast (see the section on the use of opsonizing reagents below). Count the number of BioParticles per mL with a hemocytometer using either phase-contrast or fluorescence microscopy. In most phagocytosis experiments, the number of fluorescent bacteria or yeast used is adjusted relative to the number of leukocytes. Usually, one uses between 1 and 100 particles per leukocyte, so it is important to have an accurate and precise count of your own stock populations of bacterial or yeast BioParticles per mL. Consult the literature citations at the end of this information sheet for further information on protocols that are closest to your own application. Each type of BioParticles bacteria or yeast has been tested and observed to be phagocytosed efficiently by mammalian leukocytes (mouse J774a.1 monocyte/macrophages) when used with the appropriate opsonizing reagent as recommended.

BioParticles products are sold for research purposes only. They are not intended for use in humans or for clinical diagnosis of human diseases. The toxicological properties of BioParticles bacteria and yeast have not been fully investigated. Nonetheless, these BioParticles products are not usually considered to be pathogenic strains. For example, zymosan is derived from Baker's yeast. In addition, BioParticles bacteria and yeast have been inactivated with heat or chemical treatment.

Reconstitution and Use of Opsonizing Reagents

Reconstitute the vial of BioParticles opsonizing reagent with 0.5 mL of tissue culture–grade water. This 0.5 mL volume is sufficient for opsonizing 10 mg of BioParticles bacteria or yeast.

To opsonize BioParticles bacteria or yeast, mix equal volumes of the reconstituted opsonizing reagent and 20 mg/mL suspension of BioParticles bacteria or yeast particles (reconstituted as described in *Reconstitution and Use of BioParticles Fluorescent Bacteria and Yeast*). Vortex the suspension. Incubate at 37°C for one hour. Wash 2–3 times with PBS (without sodium azide) using low-speed centrifugation (800–1500 × g, 15–20 minutes, 4°C) to form loosely packed pellets. This washing removes the excess antibody and azide from the BioParticles bacteria and yeast particles. Depending on your own experimental requirements (e.g. cell type, incubation conditions and detection system), determine the concentration of opsonized BioParticles bacteria or yeast by counting in a hemocytometer and adjust the concentration so the ratio of bacteria or yeast to leukocytes will be between 1 and 100.

References

1. J Immunol Methods 142, 31 (1991); **2.** J Immunol Methods 112, 99 (1988); **3.** J Clin Microbiol 30, 2071 (1992); **4.** J Immunol Methods 121, 203 (1989); **5.** J Immunol 133, 3303 (1984); **6.** J Immunol Methods 123, 259 (1989); **7.** J Immunol Methods 116, 235 (1989); **8.** J Immunol Methods 170, 117 (1994); **9.** Cytometry 12, 687 (1991); **10.** J Cell Biol 118, 1371 (1992); **11.** Diagn Clin Immunol 5, 62 (1987); **12.** J Insect Physiol 40, 1045 (1994); **13.** J Immunol Methods 162, 1 (1993).

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

Cat #	Product Name	Unit Size
E-2870	*Escherichia coli* BioParticles® opsonizing reagent	1 U
E-13231	*Escherichia coli* (K-12 strain) BioParticles®, Alexa Fluor® 488 conjugate	2 mg
E-23370	*Escherichia coli* (K-12 strain) BioParticles®, Alexa Fluor® 594 conjugate	2 mg
E-2864	*Escherichia coli* (K-12 strain) BioParticles®, BODIPY® FL conjugate	10 mg
E-2861	*Escherichia coli* (K-12 strain) BioParticles®, fluorescein conjugate	10 mg
E-2862	*Escherichia coli* (K-12 strain) BioParticles®, tetramethylrhodamine conjugate	10 mg
E-2863	*Escherichia coli* (K-12 strain) BioParticles®, Texas Red® conjugate	10 mg
S-2860	*Staphylococcus aureus* BioParticles® opsonizing reagent	1 U
S-23371	*Staphylococcus aureus* (Wood strain without protein A) BioParticles®, Alexa Fluor® 488 conjugate	2 mg
S-23372	*Staphylococcus aureus* (Wood strain without protein A) BioParticles®, Alexa Fluor® 594 conjugate	2 mg
S-2854	*Staphylococcus aureus* (Wood strain without protein A) BioParticles®, BODIPY® FL conjugate	10 mg
S-2851	*Staphylococcus aureus* (Wood strain without protein A) BioParticles®, fluorescein conjugate	10 mg
S-2859	*Staphylococcus aureus* (Wood strain without protein A) BioParticles®, unlabeled	100 mg
Z-2850	zymosan A BioParticles® opsonizing reagent	1 U
Z-23373	zymosan A (*S. cerevisiae*) BioParticles®, Alexa Fluor® 488 conjugate	2 mg
Z-23374	zymosan A (*S. cerevisiae*) BioParticles®, Alexa Fluor® 594 conjugate	2 mg
Z-2844	zymosan A (*S. cerevisiae*) BioParticles®, BODIPY® FL conjugate	10 mg
Z-2841	zymosan A (*S. cerevisiae*) BioParticles®, fluorescein conjugate	10 mg
Z-2843	zymosan A (*S. cerevisiae*) BioParticles®, Texas Red® conjugate	10 mg
Z-2849	zymosan A (*S. cerevisiae*) BioParticles®, unlabeled	100 mg

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.

Please visit our Web site — **www.probes.com** — for the most up-to-date information

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