

Sample Preparation using Buoyant Silica Microspheres

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Abstract

A significant challenge facing biomarker discovery and early detection of cancer is the need for a sensitive, robust and inexpensive way to isolate proteins, DNA, or cells for downstream applications, such as mass spectrometry, 2D-electrophoresis, PCR and fluorescent detection. We have developed a novel platform for sample preparation and detection of biomarkers from complex matrices, such as blood, serum, urine and other body fluids. The technology utilizes functionalized hollow silica microspheres to capture and purify target analytes from clinical samples, in lieu of conventional chromatography resins, filters or magnetic beads.

The silica microspheres are easily and inexpensively conjugated with proteins including NeutrAvidin™, protein A, protein G, or other attachment materials, such as biotin. The smooth and non-porous surface of the microspheres minimizes the nonspecific trapping of proteins and other contaminants.

The conjugated microspheres can be mixed with the matrix to capture the analyte, either by rotation, shaking or other methods. When mixing stops, the microspheres automatically rise to the top, along with the captured analyte. For viscous fluids, a quick centrifugation will concentrate the microspheres at the top of the fluid. Creativ's silica microspheres are a cost-effective and simple to use alternative for capturing cells, proteins and DNA.

Introduction

Hollow silica microspheres (microbubbles) are commonly used as a composite material in the construction of parts that need to be sturdy, yet light-weight. We propose a novel application for the sample preparation of various bioassays. The silica microspheres have many properties that make them attractive for use in target separation from solution which include:

- Stability;
- Relatively inert and bio-compatibility with organic matrices;
- Smooth, non-porous and non-permeable surfaces;
- Easy coupling with protein (NeutrAvidin™ and protein A/G) or organic compounds (biotin, Universal Nucleic Acid Binding Reagent (Cellex Inc.) etc.)

Herein we demonstrate that the functionalized silica microspheres can be used for isolating proteins, DNA and cells from solutions including serum.

Materials

Polydisperse hollow silica microspheres (3M) were modified to allow for covalent binding of NeutrAvidin™ (Thermo Scientific), biotin, protein A, protein G (Sigma) or Universal Nucleic Acid Binding Reagent (UNABR) (Cellex, Inc.). Reagents coupled to functionalized silica microspheres for this poster were:

- 1) goat anti mouse IgG,
- 2) biotinylated goat anti mouse IgG,
- 3) normal mouse IgG (Jackson Immunoresearch),
- 4) anti-ZAP-70 mAb 1E7.2 (Upstate),
- 5) biotinylated anti-ZAP-70 mAb 2F3.2 (Millipore),
- 6) anti-CD19 mAb 2E2B6B10 (Abcam), and
- 7) anti-T-Cell Receptor mAb T10B9 (Biolegend).

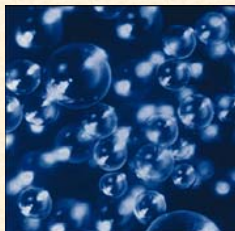
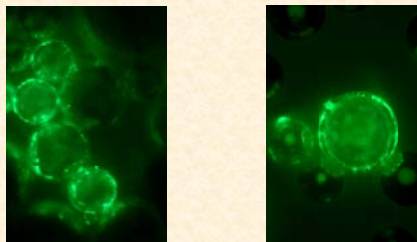


Figure 1. Unconjugated silica microspheres (3M).

Lambda DNA, cyanine dyes BOBO-1 and BOBO-3 were from Invitrogen. Dye-Doped Silica Nanoparticles were from Life Sciences Inc. Universal Nucleic Acid Binding Reagent was provided by Cellex, Inc.

Protein Capture using Protein A Conjugated Silica Microbubbles

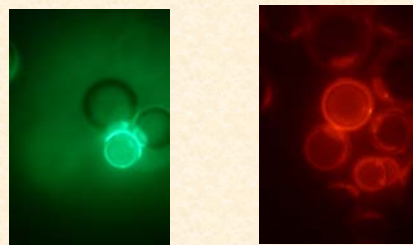


(A)

(B)

Figure 2. **Panel A.** Mouse IgG bound to silica microspheres conjugated with protein A. Mouse IgG was incubated with protein A-conjugated microspheres followed by incubation with biotinylated goat anti-mouse IgG. The bound antibody was visualized with NeutrAvidin™ conjugated to FAM Dye-Doped Silica Nanoparticle. **Panel B.** ZAP-70 bound to silica microspheres conjugated with Protein A. Mouse anti-ZAP-70 was incubated with protein A conjugated microspheres followed by incubation with Jurkat whole cell extracts. After wash, the microspheres were incubated with biotinylated mouse anti-ZAP-70 visualized with NeutrAvidin™ conjugated to FAM Dye-Doped Silica Nanoparticles.

DNA Capture using UNABR Conjugated Silica Microbubbles



(A)

(B)

Figure 3. **Panel A.** DNA bound to silica microspheres conjugated with Universal Nucleic Acid Binding Reagent (UNABR). Lambda DNA was incubated with UNABR conjugated silica microspheres and visualized with BOBO-1. **Panel B.** DNA isolated from adult bovine serum bound to silica microspheres conjugated with UNABR. Bovine adult serum was incubated with UNABR conjugated silica microspheres followed by washing and visualizing with BOBO-3.

B-Cell and T-Cell Capture using Protein A Conjugated Silica Microbubbles

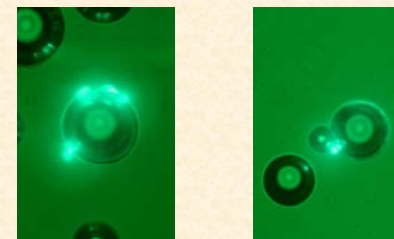


Figure 4. B-cells bound to silica microspheres conjugated with protein A. Ficoll isolated human peripheral blood mononuclear cells were incubated with protein A conjugated silica microspheres loaded with anti-CD19 mAb 2E2B6B10. Captured cells were made permeable and visualized with BOBO-10.

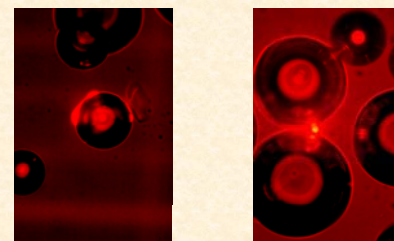


Figure 5. T-cells bound to silica microspheres conjugated with protein A. Ficoll isolated human peripheral blood mononuclear cells were incubated with protein A conjugated silica microspheres loaded with anti-T-Cell Receptor mAb T10B9. Captured cells were made permeable and visualized with BOBO-3.

Summary

Functionalized hollow silica microspheres are a cost-effective and easy-to-use alternative to conventional chromatography resins, filters or magnetic beads for preparation of clinical samples for analysis of DNA, proteins, and cells.

The silica microspheres have the following unique properties for sample preparation:

- Economical reagents
- Stable
- Relatively inert and bio-compatible with organic matrices
- Smooth, non-porous and non-permeable surface
- Easy coupling with protein or organic compounds
- No specialized equipment required
- Easy to perform assays, and
- Rapid