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Bangs Laboratories, Inc.

B E A D S • A B O V E T H E R E S T™

DESCRIPTION

The streptavidin-biotin bond is one of the strongest non-covalent, affinity interactions utilized in biological separations ($K_a = 10^{15} \text{ M}^{-1}$). As a tetrameric protein with four biotin-binding sites, streptavidin ($pI = 5$) can be covalently conjugated to functionalized microspheres with excellent retention of biotin-binding activity. Investigators have found that streptavidin-coated microspheres provide an efficient and facile means for immobilizing biotinylated antibodies and proteins, capturing biotinylated PCR products, and binding of biotinylated ssDNA or dsDNA for use in downstream applications. (For a detailed discussion of this interaction, see **Savage, D., et al.** 1992. *Avidin-Biotin Chemistry: A Handbook*. Pierce Chemical Company.)

Our streptavidin coated microspheres have been well characterized in terms of their ability to bind biotinylated molecules based on our biotin-FITC assay.

PHYSICAL PARAMETERS

Microsphere Types

| | |
|----------------------------|--|
| Polystyrene: | See the online catalog (www.bangslabs.com) for availability of specific diameters in the range of 0.02-20.0µm |
| Superparamagnetic Polymer: | Polymer / magnetite (BioMag [®] products also available. See online catalog.) |
| Two types: | <i>COMPEL™</i> - 3µm, 6µm, and 8µm <i>ProMag™</i> - 1µm and 3µm |
| Silica: | 0.15-5.0µm (density = 2.0 g/cm ³) |
| Concentration: | 10mg microspheres/mL (1% solids w/v) |
| Storage Buffer: | 100mM Borate, pH 8.5 + 0.01% BSA + 0.05% Tween [®] 20 + 10mM EDTA + ≤ 0.1% NaN ₃ (unless otherwise specified) |
| Binding Capacity: | Supplied on the Certificate of Analysis for each lot. |
| Stability and Storage: | Stable at 2-8°C for 1 year (as determined by accelerated stability testing at 22°C). |

PROCEDURE

Researchers are advised to optimize the use of particles in any application.

Preparation of Streptavidin Coated Microspheres

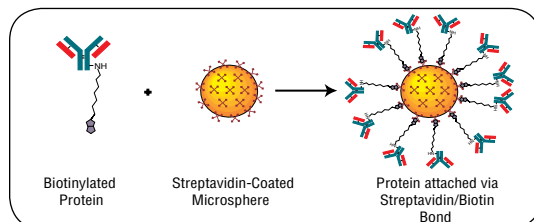
Allow microsphere suspension to come to room temperature, then vortex for approximately 20 seconds before use. Suspensions may also be rolled or rotated to ensure dispersity. A preliminary 2-3x wash should be performed to remove various additives including EDTA, anti-microbial, and surfactant. Several washing methods are possible, and a detailed description of these can be found in our TechNote 203, *Washing Microspheres*.

Attachment of Biotinylated IgG / Elution of Purified Antigen

Reagents

- Streptavidin-coated microspheres (supplied at 1% solids)

- Wash buffer (0.1 M PBS, pH 7.4)
- Elution buffer (0.1 M glycine-HCl, pH 2.5)

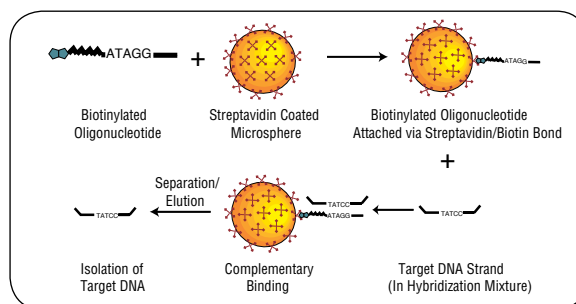


Procedure

1. Wash an aliquot of particles (1-3 times) with a 10X volume of wash buffer.
2. Resuspend the final pellet in wash buffer to a concentration of 0.05% solids (0.5 mg/mL).
3. To this solution, add your biotinylated IgG that has been dissolved in the same buffer. The protein concentration will have to be optimized, but can be based on the binding capacity of the microspheres, as reported on the Certificate of Analysis for each lot.
4. Incubate at room temperature (22°C) for 30 minutes with gentle mixing.
5. Wash the particles 3 times with another 10X volume of wash buffer.
6. Resuspend antibody-coated beads in 0.1 M PBS, pH 7.4, to desired storage concentration (often 0.5 mg/mL).
7. If using these microspheres for affinity separation of a particular antigen from a heterogeneous mixture, the bound antigen can be eluted and purified by suspending microsphere/antibody/antigen conjugate in elution buffer.

Attachment of Biotinylated Oligonucleotide

A common application of streptavidin coated microspheres in molecular biology is to separate nucleotides of interest from solution. By attaching a biotinylated oligonucleotide (A), one has a probe that is easy to manipulate and can be used for a number of applications, such as that shown in (B).^{1,2,3,4} The following protocol was developed using 1µm polymer streptavidin-coated microspheres and may be adapted for other compositions and diameters.



Reagents

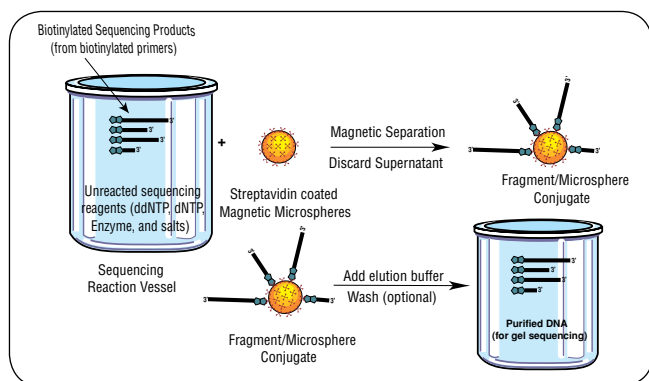
- Streptavidin-coated microspheres
- Binding/Wash Buffer: 20 mM Tris, pH 7.5; 1 M NaCl; 1 mM EDTA; 0.0005% Triton™ X-100 (520µL per binding reaction)
- Elution Buffer (as needed): 0.15M NaOH (150µL per reaction)

Procedure

1. Aliquot 100µL streptavidin coated microspheres into a microcentrifuge tube.
2. Wash 2 times in 100µL Binding / Wash Buffer by centrifuging the 1µm streptavidin coated microspheres at 10K rpm for 3 minutes, and decanting the supernatant.
3. Re-suspend the microspheres in 20µL Binding / Wash Buffer and add 5-10µg biotinylated ds or ss oligonucleotide. Keep bead concentration in the range of 10-50 mg/mL during the binding step.
4. Incubate 15 minutes at room temperature on a vortexer (Setting 1), then centrifuge and decant the supernatant. *Note:* If an adjustable vortexer is unavailable, intermittent manual mixing is advised.
5. Remove any unbound biotinylated oligonucleotide from the streptavidin-oligo beads by washing 2 times in 100µL Binding / Wash Buffer.
6. Re-suspend in 100µL Binding / Wash Buffer. Oligo-bound microspheres are now ready for downstream applications.

Purification of DNA Sequencing Reactions

A fundamental aspect of modern molecular biology is DNA sequence analysis. In order for the DNA to be accurately sequenced, and the background noise reduced, it is necessary to first remove impurities that are remnants of the sequencing reaction (enzyme, salts, unreacted dye terminators, etc.). The following procedure outlines how streptavidin coated magnetic microspheres can simplify this process. Standard protocols exist for DNA sequencing.^{3,5} Automated sequencing instrumentation is offered by Life Technologies (ABI PRISM®) and Amersham Life Sciences (Thermo Sequenase dye terminator cycle sequencing kit), among others. Therefore, this protocol is specific to using streptavidin coated superparamagnetic microspheres to purify and isolate DNA for gel sequencing. The reaction is as follows:



Reagents

- Streptavidin-coated paramagnetic microspheres (supplied at 1% solids)
- Binding Buffer: 1X TES (10 mM Tris-HCl; 1 mM EDTA; 1 M NaCl, pH 8.2) and 0.2% Tween® 20
- Wash Buffer: 1X TES buffer
- Elution Buffer: 10 mM EDTA, 95% formamide, 0.05% bromophenol blue (or other DNA stain)

Procedure

1. Add 20µL of microspheres and 20µL of binding buffer to each reaction vessel (per 50µL volume). *Note:* These volume ratios are a baseline that might need to be optimized for your particular sequencing reaction.
2. Incubate for 15 minutes with gentle mixing.
3. Wash 2 times (by magnetic separation) with 10µL of wash buffer, and 1 time with 10µL of H2O.
4. Elute DNA by resuspending in 6.5µL of elution buffer at 90°C for 5 minutes with mixing.

REFERENCES

1. Fry, G., et al. 1992. *BioTechniques*, 13(1);124-131.
2. Tong, X., L.M. Smith. 1992. *Analytical Chemistry*, 64(22);2672-2677.
3. Fujita, R., A. Swaroop. 1995. *BioTechniques*, 18(5);796-800.
4. Ji, H., L.M. Smith. 1993. *Analytical Chemistry*, 65(10);1323-1328.
5. Hopgood, R., K.M. Sullivan, P. Gill. 1992. *BioTechniques*, 13(1);82-92.

TRADEMARKS AND REGISTERED TRADEMARKS

1. COMPEL™ and ProActive® are trademarks and registered trademarks of Bangs Laboratories, Inc.
2. ProMag™ is a trademark of Polysciences, Inc.
3. Tween® is a registered trademark of ICI Americas, Inc.
4. Triton™ is a trademark of Union Carbide Corporation.
5. ABI PRISM® is a registered trademark of Life Technologies.

STORAGE AND STABILITY

Store at 2-8°C. Freezing of particles may result in irreversible aggregation and loss of binding activity.

SAFETY

This particle suspension contains sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azides. Upon disposal of material, flush with a large volume of water to prevent azide accumulation. Please consult the Material Safety Data Sheet for more information.

This product is for research use only and is not intended for use in humans or for *in vitro* diagnostic use.

ORDERING INFORMATION

| Cat. Code | Description | Sizes |
|-----------|---|------------------------|
| UMC3N | COMPEL™, 3µm and 6µm | 0.5g or 1.5g |
| UMC4N | COMPEL™, 8µm | 0.5g or 1.5g |
| PMS1N | ProMag™ 1 Series • Streptavidin | 1mL, 2mL, 5mL, or 25mL |
| PMS3N | ProMag™ 3 Series • Streptavidin | 1mL, 2mL, 5mL, or 25mL |
| CM01N | ProActive® Magnetic Streptavidin Microspheres | 1mL, 2mL, 5mL, or 10mL |
| CP01N | ProActive® Polymeric Streptavidin Microspheres | 1mL, 2mL, 5mL, or 10mL |
| CP01* | ProActive® Dyed Polymer Streptavidin Microspheres | 1mL, 2mL, 5mL, or 10mL |
| | * See our website for color chart | |
| CS01N | ProActive® Silica Streptavidin Microspheres | 1mL, 2mL, 5mL, or 10mL |

Order online anytime at www.bangslabs.com.