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BEADS ● ABOVE THE REST™

Description

The avidin/biotin interaction is one of the strongest non-covalent bonds ($K_a=10^{15}/M$ vs. $10^7-10^{11}/M$ for antibody-antigen interactions). This complementarity, combined with the small size of biotin (MW=244.3), yields an ideal system for affinity binding, with numerous applications in areas such as immunology and cell/molecular biology.

Although our microspheres are designed as solid support with maximal binding and minimal nonspecific binding, this avidin/biotin system does have its limitations. Avidin's carbohydrate moieties can cause nonspecific interactions with many proteins. Also, avidin's pI of approximately 10 (a net positive charge at neutral pH) can result in nonspecific binding of negatively charged ligands, such as nucleic acids.

One solution to this is to use streptavidin, a tetrameric protein with four biotin binding sites that is similar to avidin in its molecular structure, yet lacks the carbohydrates that can result in nonspecific interactions. Streptavidin's pI of approximately 5 (a net negative charge at neutral pH) avoids nonspecific charge interactions with biotin coated microspheres.¹

Our biotin coated microspheres have been fully characterized in terms of their ability to bind free avidin, as determined by the HABA assay², and therefore will require minimal optimization when determining the correct concentration of ligand to be bound.

Physical Parameters

Microsphere Types

Polystyrene:	0.02µm to 1mm, plain or dyed in a variety of colors, including fluorescent
Superparamagnetic Polystyrene:	Polydisperse polystyrene/magnetite with nominal mean diameter of ~1µm
Two types:	<i>Classical</i> , with magnetite exposed at surface, and <i>Encapsulated</i> , with outer polymer shell.
	Magnetite percentage ranges from 12-66% by weight (density ranges from 1.16-2.24 g/cm ³).
Silica:	0.15-5.0µm (density=1.96 g/cm ³)
Concentration:	10mg microspheres/mL (1% solids w/v)
Storage Buffer:	100 mM Borate, pH 8.5 + 0.01% BSA + 0.05% Tween 20 + 10 mM EDTA + 0.1% NaN ₃ (unless otherwise specified)
Binding Capacity:	Supplied on the Certificate of Analysis for each lot.

Procedure

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

Preparation of ProActive Biotin Coated Microspheres

Allow microsphere suspension to come to room temperature, then vortex for approximately 20 seconds before use. A preliminary wash is necessary with most applications, to remove various additives including EDTA, antimicrobials, and surfactants. Several washing methods are possible, and a detailed description of these can be found in our TechNote 203, "Washing Microspheres."

Attachment of a Streptavidin-tagged IgG

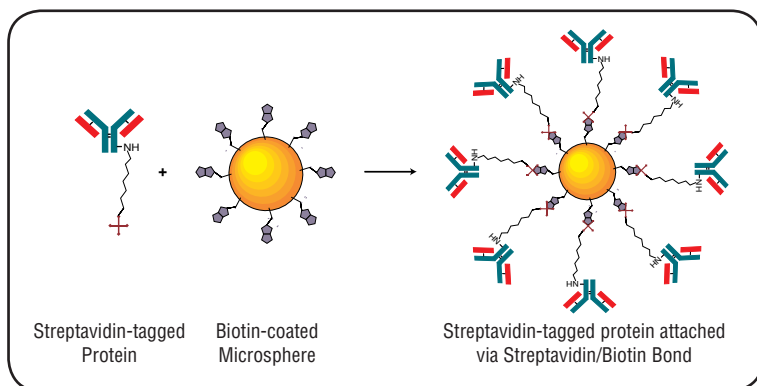
Reagents

- Biotin coated microspheres (supplied at 1% solids)

- Wash/storage buffer (0.1 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 streptavidin-tagged IgG that is dissolved in the same buffer. The protein concentration will have to be determined empirically, 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 (18-25°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. *Optional:* If using these antibody-coated microspheres for affinity separation of a particular antigen from a heterogeneous mixture, the bound antigen can be eluted and purified by suspending the microsphere/antibody/antigen conjugate in elution buffer.



Note: Separate polymeric and silica microspheres via centrifugation, and with a magnet for superparamagnetic microspheres.

References

1. **Savage, D., et al.** 1992. *Avidin-Biotin Chemistry: A Handbook*. Pierce Chemical Company.
2. **Hermanson, G.** 1996. *Bioconjugate Techniques*, Pierce Chemical Company: Academic Press, 591-592.

Storage and Stability

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

Safety

The Storage Buffer may contain 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

Catalog Code	Description	Sizes
CP10N	ProActive® Polymeric Biotin Microspheres	1mL, 2mL, 5mL, or 10mL
CP10*	ProActive® Dyed Polymer Biotin Microspheres	1mL, 2mL, 5mL, or 10mL
	* See our website for color chart	

Order online anytime at www.bangslabs.com.