

Power-Bind™ Streptavidin Coated

Microparticles
March 2005

Introduction

We have developed a series of streptavidin coated (SA-MP) microparticles to improve and simplify the binding of ligands to microparticles. Power-Bind™ SA-MP combine the advantages of high surface area and easy, high affinity, high specific activity binding. Here, the properties of Power-Bind SA-MP are reviewed and some data on diagnostic applications are given. They can also be used in a variety of molecular biology applications.

Seradyn Power-Bind SA-MP are monodisperse particle suspensions with streptavidin covalently bound to the surface (of OptiLink® carboxylate-modified particles) in a highly active form. The use of microparticles as a solid phase support in various immunoassays and affinity purifications has been known for some time. Achieving high activity and stable binding of solid phase ligands has been a major difficulty. Standard protocols are available to biotinylate a wide range of analytes including proteins, nucleic acids, and haptens. Biotinylated compounds become stably bound to Power-Bind SA-MP after simple incubation in buffer. This is due to the high affinity of the biotin-streptavidin interaction. SA also functions as a spacer which improves the specific activity of the bound ligand.

Benefits

Power-Bind SA-MP addresses the concerns of diagnostic test kit manufacturers and nucleic acid researchers. With well known biotin-streptavidin reactions, scientists can now easily bind ligands to microparticle surfaces.

- Dissociation constant (K_d 10^{-15} molar)
- Stably bound ligands
- Easy one-step binding protocols for biotinylated ligands
- High activity of surface bound ligands
- Easily solve difficult coupling problems
- Simple aqueous biotinylation reactions
- Low nonspecific interactions (fully blocked microparticle surface)
- Highly mobile particles for membrane-based applications
- Can be used in EIA formats with biotin/enzyme detection systems
- Beneficial spacer effect of SA molecule
- Choice of 0.3 μ M or 0.8 μ M diameters for different applications
- High biotin-binding capacity for molecular biology applications

Background

Solid phase reactants are widely used in immunoassay and affinity chromatography.¹ The immobilization of reactants permits easy and effective separation of analytes from complex mixtures. Immobilized reagents are generally very stable; this allows for prolonged storage. With solid phases such as microplates and membranes, enzyme immunoassays (EIA) have proliferated in recent years. Membranes have become increasingly useful supports for EIA and related techniques.² Particulate supports such as agarose and acrylamide particles have been most widely used for affinity chromatography.

Microparticles are polymeric spheres with diameters ranging from about 50 nm to 2 μM . MP suspensions have extremely high surface area which can greatly enhance the kinetics of solid phase reactions. For example, the surface area of one mL of 0.3 μM particles at 1% solids is almost 2000 cm^2 . The most common polymer type is polystyrene (PS-MP). PS-MP may be modified to have surface carboxyl or amino functions which are suitable for covalent coupling. Due to their small size and surface charge, MP generally remain stably suspended and thus are suitable for batch purifications and immunoagglutination reactions. In addition, dyed MP are increasingly being used as indicators in membrane-based assays.

When working with solid phase reactants, there are several important considerations: surface area available, reactivity and stability of bound reagents, and ease of coupling. Surface area has a major effect on the sensitivity and kinetics of EIA. Microplates have limited surface area which results in slow reactions. Membranes provide higher surface area and capacity by permitting reactions to occur throughout the thickness of the support. Microparticles can provide orders of magnitude more surface area, depending on the particle size selected.

Reactivity and stability of solid phase reactants may vary with the mode of attachment to the support. Proteins may be immobilized by adsorption to hydrophobic surfaces. Adsorbed proteins suffer some loss of activity and may be eluted during prolonged storage or washing with detergent. Covalent coupling results in more stably bound reactants, but as with adsorption there will be loss of activity.³ The use of protein spacers has been proposed as a means of increasing both the stability and the specific activity of bound solid phase reactants.⁴

Coupling of proteins to solid phases may be accomplished by a variety of protocols. Seradyn has available a technical bulletin describing simple but effective methods for coupling of proteins to MPs.⁵ However, some analytes such as haptens or nucleic acids are difficult to attach directly to the surface of MPs. The small size of haptens results in low activity after direct binding to MP surfaces. Nucleic acids bind poorly to negatively charged MPs because of charge repulsion.

One solution to these problems is the use of a biological binding system. The streptavidin-biotin binding interaction is widely used in immunoassay and affinity chromatography.⁶ SA is a tetrameric protein of

60 kD which binds up to four molecules of biotin with an association constant of about 10^{15} M. The biotinylation of many compounds including proteins, haptens and nucleic acids is easily accomplished and the biotinylated species will then bind to Power-Bind™ SA-MP. The resulting conjugate is stable, has high specific activity, and provides high surface area on which reactions can proceed.

Product Description

All particles used for Power-Bind SA-MP are produced by Seradyn using proprietary manufacturing processes to insure maximum quality and reliability. These particles are designed for various applications such as diagnostics assays, biomedical research, nucleic acids research, and large scale affinity purifications. Please ask for current inventory listing.

- Streptavidin covalently bound to carboxylate modified PS-MP
- Nominal particle diameters of 0.3 μ M and 0.8 μ M
- Packaged at 1.0% solids
- Monodisperse, colloiddally stable particle suspensions
- 24 month stability of at 4 °C

Properties

The choice of SA over avidin was based on the physicochemical properties of these proteins. Avidin has an isoelectric point of 10.5 and is highly glycosylated; this results in considerable nonspecific binding. SA, a nonglycosylated protein with pI of 6.1, has low nonspecific binding.

SA is covalently attached to carboxylate-modified microparticles (CM-MP). After coupling of SA, the SA-MP are stabilized by the addition of non-reactive detergent.

Power-Bind SA-MP are monodisperse (colloiddally stable) reagents. This is established by submitting a dilute suspension of the particles in buffer to size analysis on a Coulter model N4+ Quasielastic Light Scattering instrument (QELS). Any aggregation would be picked up by this method. SA-MP preparations are more stable as the pH increases above the pI of SA, because the protein takes on an increasingly negative charge. SA-MP remain monodisperse at pH greater than 7.0 with 0.1 % Tween-20 in the buffer. SA-MP are compatible with a variety of buffers including phosphate, TRIS and MOPSO.

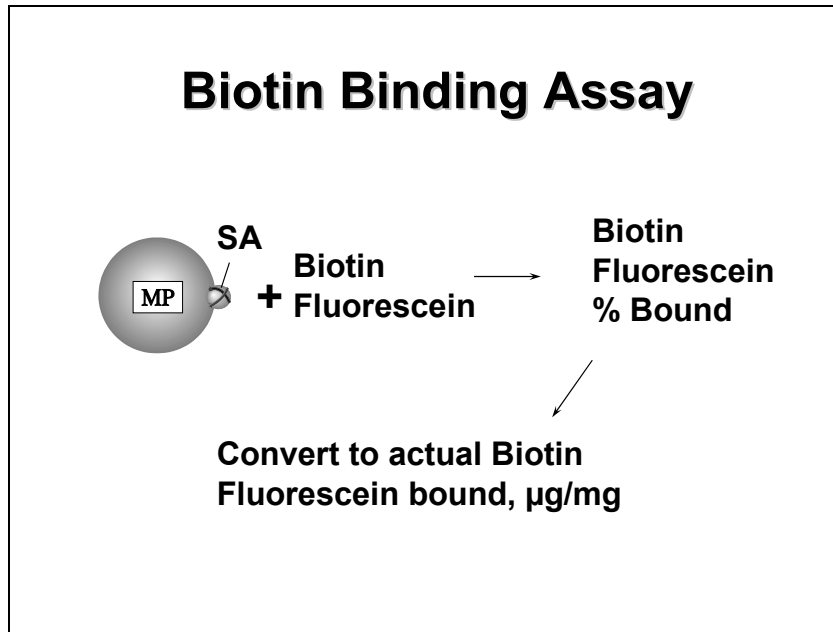
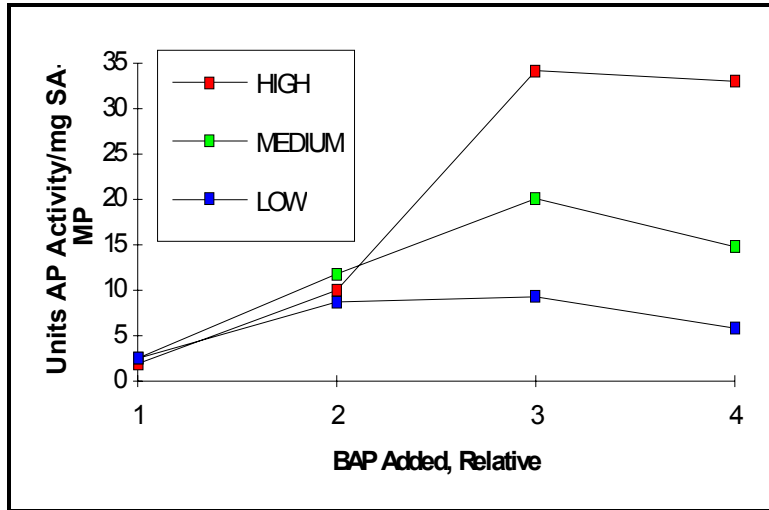


Fig. 1 Biotin-binding capacity measured by binding of biotin fluorescein

We currently assay the biotin binding activity of SA-MP using biotinylated fluorescein (BF). SA-MP binds BF in proportion to the amount of immobilized SA. The BF binding capacity increases with decreasing particle diameter; this is due to the increased surface area of smaller diameter particles. The decrease in bound BF activity at very high levels of applied BF may be related to the steric effects of close clustering of the captured BF. Note that BF is applied in large excess over the SA-MP binding capacity so that saturation of bound SA occurs under conditions of the assay.

Biotin-Binding Capacity



Streptavidin-Microparticle binding profile for biotinylated-alkaline phosphatase (BAP)

Prior to our current biotin fluorescein (BF) assay, we assessed biotin-binding capacity with a biotinylated alkaline phosphatase system (BAP). SA-MP (0.284 μ M at 100 mg/ml solids of various sizes) were incubated for one hour at room temperature with 5-fold increasing dilutions of BAP (BMB 1119-834). The buffer contained: sodium phosphate, 50 mM, pH 7.4; sodium chloride, 100 mM; BSA, 1.0%; and sodium azide, 0.1% (PNBA buffer). Reactions were centrifuged and washed twice with PNBA and resuspended to 50 μ g/ml solids. Alkaline phosphatase activity was measured on a Cobas Bio with p-nitrophenyl phosphate as substrate.

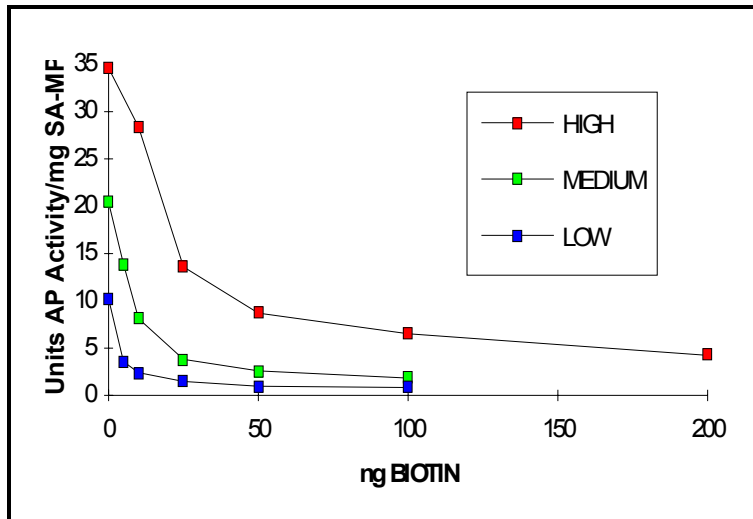


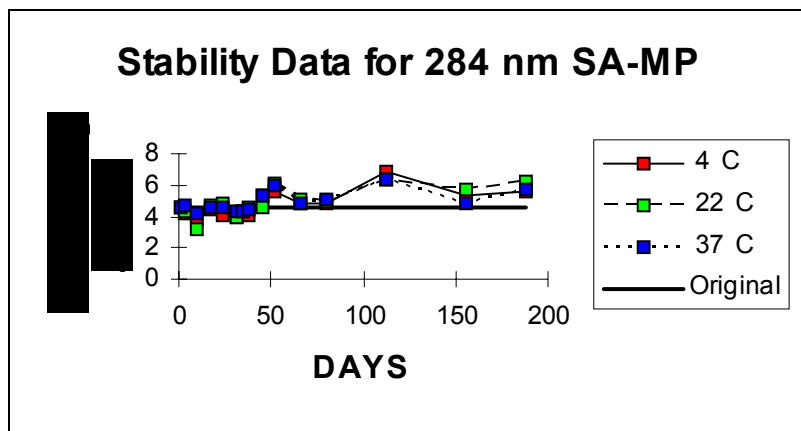
Fig. 2 Biotin inhibition of biotin alkaline phosphatase binding to SA-MP

Stock solution of d-biotin was prepared in DMSO and diluted to various concentrations in phosphate buffer. SA-MP were incubated with the biotin dilutions for 15 min and then excess BAP was added in PNBA buffer and binding activity was determined.

Specificity of Binding

The specificity of binding of BAP to the particle surface was evaluated in two ways. First, as seen in Fig 2, the binding was inhibited by free biotin. Second, the binding of nonbiotinylated AP was measured and found to be negligible. Taken together, these data indicate that the MP surface is fully blocked to adsorptive binding of proteins. When binding low molecular weight compounds to SA-MP, the possibility of interaction with the blocking BSA should be considered since BSA binds a variety of small molecules. The use of gelatin-blocked SA-MP may be preferable in such situations.

Stability Data



Stability of SA-MP as determined with biotinylated alkaline phosphatase system (BAP)

Aliquots of Power-Bind SA-MP were stored at 4 °C, 22 °C and 37 °C. At various times the BAP binding activity of these aliquots was assayed.

Stability of Power-Bind SA-MP

The stability of SA-MP under heat stress is shown in above. The activity remained constant for 188 days at 37 °C indicating excellent stability. This demonstrates that there was no detectable elution of SA from the particle surface. SA is known to be a robust protein, withstanding a variety of denaturing conditions.⁶ We have also found that sonication of SA-MP or SA-MP with bound BAP is harmless.

General Usage Conditions

- For binding reactions, use buffers with pH >7.0 (phosphate, tris, MOPSO, and similar buffers)
- Maintain salt concentration of 100-150 mM and blocking protein (BSA or gelatin) at 1%
- Mix SA-MP and biotinylated ligand for approximately 1 hour at RT
- Maintain an excess of biotinylated ligand to prevent crosslinking through multiple biotin-SA bonds
- Alternatively, where ligand is limited, titer with free biotin to prevent crosslinking
- After binding, wash with buffer containing 1% BSA or gelatin
- Wash by either centrifugation or tangential flow filtration methods
- Sodium azide may be used as a preservative (0.02 to 0.1%)

Applications of Power-Bind

Standard protocols are available for the biotinylation of numerous compounds including nucleic acids, proteins and haptens.⁵ Biotinylation reactions may be carried out with commercially available activated biotin derivatives including active ester and hydrazide. Compounds which are difficult to attach to MP surfaces by conventional means may be amenable to biotinylation; this includes compounds for which activation reactions must be performed in organic solvent. In the latter case, biotinylation may be carried out in organic solvent; then the biotin derivative is simply mixed with the SA-MP. Nucleic acids, which adsorb poorly to MP surfaces, are readily bound to SA-MP after biotinylation.

Agglutination assays are the most common application of PS-MP. Avidin-MP have been used to prepare reagents for slide agglutination.⁷ In this work it was found that relatively crude viral extracts were suitable for sensitizing avidin-MP, whereas more highly purified antigen was required to achieve an acceptable assay when using direct adsorption of antigen onto PS.

Affinity purification is another application for Power-Bind SA-MP. DNA binding proteins have been isolated using magnetic SA-coated beads.¹² A biotinylated DNA fragment containing the binding sequence for transcription factor was immobilized to the magnetic beads. The resulting beads were incubated with partially purified cellular extracts and the bound transcription factor was eluted with

ELISA Formats

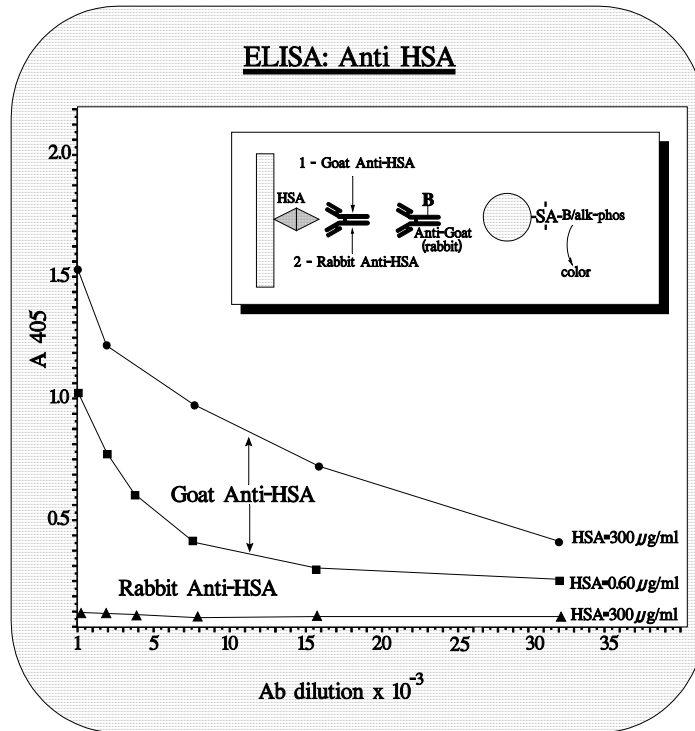
high salt. Power-Bind SA-MP provides an attractive alternative to the magnetic beads. The high surface area and biotin binding capacity of the smaller (0.15 to 0.3 μM) SA-MP are advantageous in this application. The particles with attached biotinylated molecules can be separated from the mixture by centrifugation.

MP coated with antibody have been used in ELISA to achieve an amplification of signal.¹¹ We have performed model studies using SA-MP in an ELISA format, Fig.4. Microplate wells were coated with human serum albumin (HSA) and then reacted in succession with goat anti-HSA, biotin anti-goat and SA-MP. Detection was then done with BAP.

The specificity of binding of SA-MP to the sensitized plates was excellent; controls in which biotinylated antibody was omitted gave very low readings. Control reactions in which rabbit primary antibody was substituted for the goat antibody also gave low readings. Wells coated with decreasing concentrations of HSA gave proportionally lower binding. Control of nonspecific binding was improved by using high salt, detergent and sodium salicylate in the wash buffer. Sodium salicylate appears to function by adsorbing to the polystyrene surface of the plate which increases the negative surface charge. The use of SA-MP in the range of 0.01 to 0.1% gave excellent binding in the ELISA system.

In this preliminary ELISA work, BAP was added in a separate step. . We have also demonstrated that dyed SA-MP can be captured in coated microplate wells and on membranes, allowing direct visual detection of immobilized biotin species.

Applications of Power-Bind



Antibody detection in microplates using SA-MP

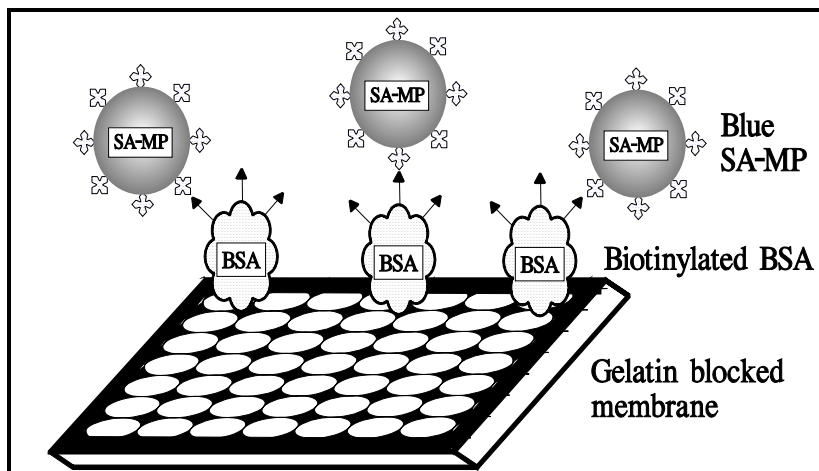
Dynatech Immulon II plates were coated with various concentrations of human serum albumin (HSA) in sodium phosphate, 50 mM, pH 7.4; NaCl, 100 mM; sodium azide, 0.1%. Plates were then blocked with the same buffer containing 1% ovalbumin (PNOA). Fractionated anti-HSA polyclonal antibodies from goat or rabbit (Sigma cat. no. A1151 and A0659, respectively) were diluted serially in PNOA buffer and applied to the HSA coated wells. Further steps were performed using a buffer containing: sodium phosphate, 50 mM, pH 7.4; NaCl, 100 mM; ovalbumin, 1.0%; sodium azide, 0.1%; tween 20, 0.1%; and sodium salicylate, 100 mM. Plates were washed and incubated for 15 min. with this buffer to decrease nonspecific binding. Biotin anti-goat antibody (affinity purified rabbit polyclonal, Sigma B7024) diluted 1:1000 in wash buffer was applied to all wells. After washing, SA-MP diluted to 0.1% solids was applied to all wells, incubated and washed. Biotin alkaline phosphatase (BAP) (1000 U/ml, BMB 1119834) diluted 1:1000 was then applied to all wells. After a final washing with wash buffer, alkaline phosphatase substrate (p-NPP) was added and allowed to react for 5 min before terminating reactions with 1.0 M NaOH. Absorbance was read at 405 nm using a Dynatech MR700 plate reader.

The binding of BAP to SA-MP can be inhibited by preincubation with free biotin, Fig. 2. From these biotin inhibition curves the effective biotin binding capacity of SA-MP may be calculated. The biotin binding capacity determined in this way is likely to be lower than for methods using radiolabeled biotin; this is because the accessibility of binding sites to free biotin is certainly greater than to BAP.

Membrane Formats

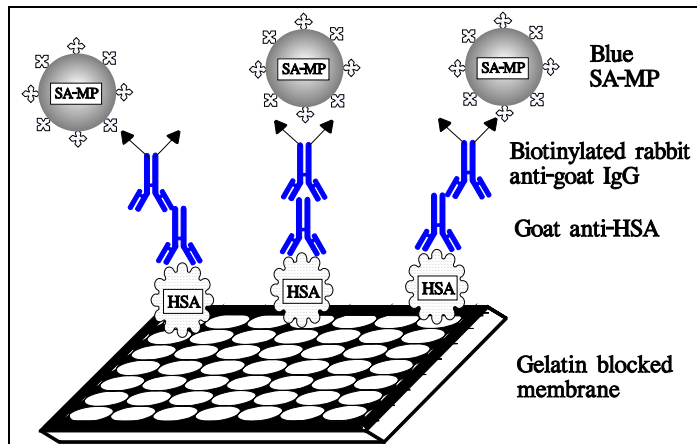
Recently, formats in which MPs are used in conjunction with membranes have become popular. Membranes of suitable pore size may be used to detect the agglutination of sensitized MP.⁸ The retention of agglutinated particles is detected directly if dyed MP are used. The Abbott IMx system uses a method called microparticle capture enzyme immunoassay (MEIA).⁹ MEIA is run in either sandwich or competitive mode. In either case the MP suspension is first reacted with the sample and is then adsorbed onto a glass fiber membrane. Detection is via enzyme conjugates with fluorescent substrates. A particularly interesting test mode involves the capture of dyed MP by immobilized antibody.¹⁰ MP are sensitized with a first antibody and after exposure to the sample are allowed to migrate horizontally through a membrane past an immobilized line of second antibody. If analyte is present in the sample, the MP are captured to give a colored line.

Membrane based tests, such as described above, represent a fertile area for applications of SA-MP. The binding of compounds through SA should improve specific activity by the spacer effect.⁴ This will be an important advantage in situations where high specific activity of bound reagents is critical. The cost per test is expected to be low for membrane tests due to the small consumption of MP; this improves the practicality of developing commercial membrane based assays using SA-MP.



We have demonstrated specific capture of blue-dyed SA-MP on membranes. Schleicher and Schuell AE98 membranes were sensitized with biotinylated-BSA (b-BSA) and blocked with gelatin. Using specially buffered blue SA-MP as the only reagent, we were able to detect 10 to 20 pg b-BSA.

Membrane Formats



The same membranes were sensitized with human serum albumin (HSA) and blocked with gelatin. Goat anti-HSA antibody (Sigma A1151) was captured, followed by biotinylated rabbit anti-goat IgG (Sigma B7024). Washes were performed with a buffer containing Tween 20. Again, blue SA-MP were used as the detection system. This system was able to detect a 1:100,000 dilution of the goat anti-HSA.

Summary

- Highly active SA-MP prepared and characterized
- Binding is limited to biotin-SA interaction
- Detection in ELISA and membrane formats demonstrated
- SA-MP provides an alternative for streptavidin enzyme conjugates
- Variety of biotinylated compounds may be immobilized to SA-MP
- High activity "spacer effect" of the bound SA
- Excellent stability of Power-Bind SA-MP

Ordering Power-Bind™ Microparticles

Power-Bind Streptavidin Microparticles

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

Power-Bind Streptavidin	Vol.	Catalog No.	Binding Capacity
0.3 μM	1 mL	2900-0701-011150	~1200 pmol/mg
0.3 μM	5 mL	2900-0701-010150	~1200 pmol/mg
0.3 μM	15 mL	2900-0701-010250	~1200 pmol/mg
0.8 μM	1 mL	2900-1701-011150	~1200 pmol/mg
0.8 μM	5 mL	2900-1701-010150	~1200 pmol/mg
0.8 μM	15 mL	2900-1701-010250	~1200 pmol/mg

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