How to Immobilize and Produce the Strongest Signals Without Background Noise

Introduction

The use of immobilized streptavidin on microwell plates and beads both non-magnetic and magnetic is prolific in scientific assays. The binding constant of biotin to streptavidin, $>10^{-15}$ M^{-1}, is near covalent and the kinetics of binding is extremely fast making the biotin-streptavidin pair ideal for bioassays on streptavidin immobilized surfaces. It has been shown that biotinylated biomolecules (*i.e.* antibodies, peptides, and oligonucleotides) immobilized on streptavidin surfaces retain their binding affinity.

Streptavidin-coated magnetic beads, with their fast magnetic response time, have particular utility due to their ease of processing, leading to their use in many high throughput and multiplexed assays. Steptavidin-coated magnetic beads have been incorporated in automated platforms such as the KingFisher from ThermoFisher, BioMek Systems from Beckman Coulter and the Tecan Genesis from Tecan AG.

Biotinylated proteins and oligonucleotides, in association with streptavidin-coated magnetic particles, are being used in clinical diagnostic assays, proteomic and genomic assays including:

- Immunoassays/Immunodiagnostics
- Purification of DNA/RNA binding proteins
- Protein purification
- Biopanning
- Cell isolation
- Gene sequencing

Optimizing the Assay

Assay developers are faced with two over-riding considerations when developing a streptavidin magnetic bead based assay; optimizing the assay for the highest signal and for the lowest non-specific binding (NSB). For optimization it is important to consider biotin-binding capacity.

How can these assays be optimized?

Increase the biotin-binding capacity:
Magnetic beads produce non- specific binding and therefore background noise. By increasing binding, the signal on each bead can be greatly increased. As biotin-binding capacity increases less beads are required resulting in less background from the use of less beads. Higher capacity leads to reduced background and a more sensitive assay.

Increase the surface area of the beads:
Small sized beads have greater surface area resulting in increased loading of streptavidin and therefore will lead to higher signal per bead. Many sizes of bead exist, the most popular size being 2.8µm. Therefore, a bead that has a smaller diameter will allow for much better signal.

Indirect detection or capture assays do not require uniformed sized beads: Assays which employ enzyme-mediated signal generation, like ELISA, require high biotin binding capacity but not uniformed sized beads. For these types of assays it is most important that biotin-binding/mg is consistent lot-to-lot for assay reproducibility. Uniform particles are only necessary when the signal is detected directly on the bead by visual means (i.e., single molecule detection).

Current High Capacity Streptavidin Beads on the market

There are many magnetic streptavidin bead manufacturers, and the beads can all use the same magnetic platforms because at their core, they are all made from the iron magnetite material. The beads differ in their size, uniformity, magnetic response time and most import, in their capacity to bind biomolecules.

By covalently crosslinking streptavidin using a proprietary conjugation chemistry, SoluLinK is able to efficiently increase streptavidin loading with high stability and low streptavidin leaching, as shown in Figure 1. This chemistry produces the highest biotin binding capacity of any bead on the market; greater than 12 nmol biotin/mg of bead.

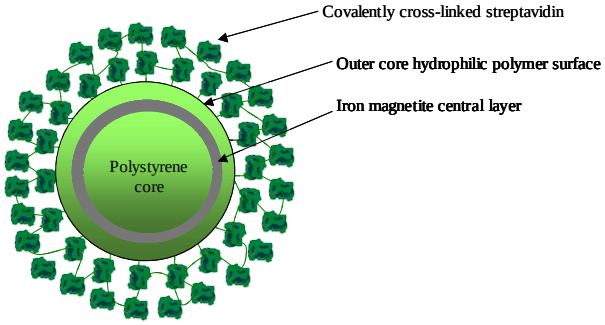

Figure 1: Schematic representation of SoluLinK's covalently crosslinked streptavidin magnetic beads.

Figure 2 compares biotin-binding capacity of SolulinK's NanoLink Streptavidin beads to the published data of other commercially available streptavidin-magnetic beads.

As described, assays have varying requirements for streptavidin-magnetic beads. NanoLink Streptavidin beads are polydispersed of average diameter of 800 nm and possess biotin binding capacity of >12 nmol/mg. While the beads can be used for a variety of applications, they are best suited for protein capture using

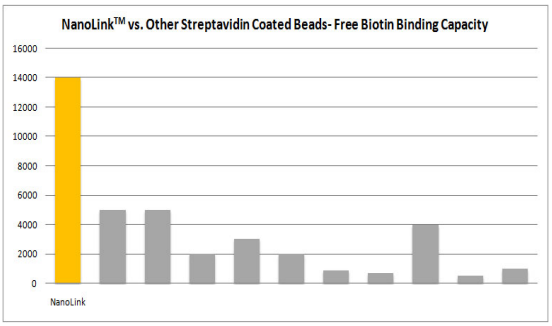

Figure 2: Comparison of SoluLink's NanoLink Streptavidin beads to the published data of other commercially available streptavidin-magnetic beads. The beads are measured in biotin binding capacity pmol/mg of bead.

biotinylated DNA, peptides or other proteins as bait.

Ultra-high capacity microspheres reduce the overall particle mass required to immobilize biotinylated proteins and oligonucleotides. This leads to reduced costs and lower non-specific background (NSB).

Demonstration of the low background binding and high biotinylated-antibody capture efficiency of the NanoLink beads is presented in Figure 3. The overlaid UV spectra on the left shows minimal non specific binding of bovine IgG to NanoLink beads. The graph on the right presents the binding of biotinylated bIgG (using SoluLink's ChromaLink Biotin Reagent) to NanoLink beads.

Conclusion

SoluLinK's NanoLink Streptavidin Magnetic Beads offer unparalled binding capacity allowing scientists to optimize the efficiency of their magnetic assays.

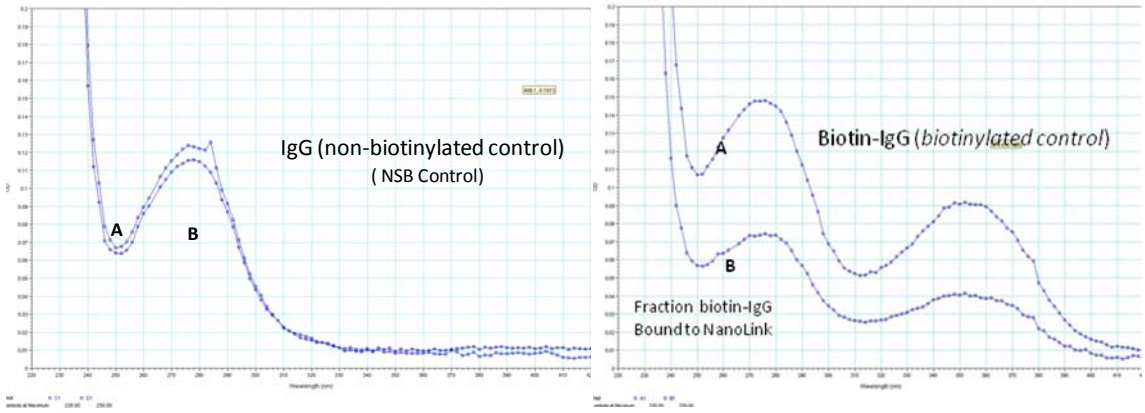

Figure 3: Left: The low non-specific binding of biotinylated IgG to NanoLink beads is demonstrated; **A** UV scan of biotinylated bovine IgG (bIgG) and **B** supernatant of bIgG and NanoLink streptavidin beads. Right: Demonstration of biotin binding capacity of NanoLink beads by addition of 250 ug biotinylated bIgG (3.4 biotins/bIgG as determined by use of ChromaLink Biotin biotinylation reagent (Solulink) to 0.5 mg of NanoLink beads. Note: SoluLink's ChromaLink Biotin Labeling Reagent has a unique absorbance signature downstream of the that can be seen in this graph.

SoluLinK offers the following resources to successfully use NanoLink Streptavidin Magnetic Beads and ChromaLink Biotin Labeling reagent:

NanoLink	Protocol: Link
ChromaLink Biotin	Protocol: Link Calculator: Link

SoluLinK Catalog Listings

Product	Size	SoluLinK Catalog No.
NanoLink Streptavidin Magnetic Beads	10 mg	M-1002-010
ChromaLink Biotin Labeling Kit	Kit- Labels up to 5mg of protein	B-9007-105K
ChromaLink One-Shot Antibody Biotinylation Kit	Kit – Labels 100µg of antibody	B-9007-009K
ChromaLink Biotin Labeling Reagent	10 mg	B-1007-110