WHITE PAPER

Streptavidin Magnetic Beads to Optimize the Signal-to-Noise Ratio

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Introduction

The use of immobilized streptavidin on microwell plates and beads, both nonmagnetic and magnetic, is prolific in scientific assays. The binding constant of biotin to streptavidin, $\approx 10^{-15}$ m, approaches the strength of a covalent bond, 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 affinities.

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 Thermo Fisher Scientific Inc., 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 overriding considerations when developing a streptavidin magnetic bead–based assay; optimizing the assay for the highest signal and for the lowest nonspecific 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 nonspecific binding and, therefore, background noise. By increasing binding, the signal on each bead can be greatly increased. As biotin-binding capacity increases, fewer beads are required, resulting in less background from the use of fewer beads. Higher capacity leads to reduced background and a more sensitive assay.

Increase the surface area of the beads

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

Indirect detection or capture assays do not require uniform sized beads

Assays that use enzyme-mediated signal generation, like ELISA, require high biotin binding capacity but not uniform 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 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 important, in their capacity to bind biomolecules.

Efficiently increase streptavidin loading with high stability and low streptavidin leaching by covalently crosslinking streptavidin using the SoluLINK(R) bioconjugation technology, 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 2 compares the biotin-binding capacity of NanoLINK Streptavidin beads to the published data of other commercially available magnetic streptavidin beads.

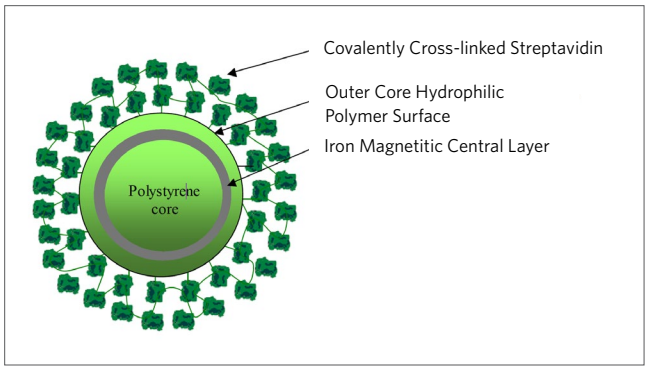

Figure 1 *Schematic representation of covalently crosslinked streptavidin magnetic beads.*

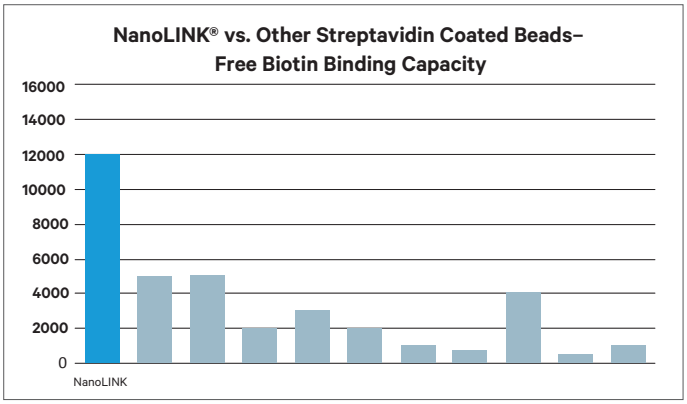

Figure 2 *Comparison of NanoLINK Streptavidin beads to the published data of other commercially available magnetic streptavidin beads. The beads are measured in biotin binding capacity pmol/mg of bead.*

As described, assays have varying requirements for magnetic streptavidin beads. NanoLINK Streptavidin beads are polydispersed, with an average diameter of 1 µm, 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 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 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 NSB of bovine IgG to NanoLINK beads. The lower graph presents the binding of biotinylated bovine IgG (using ChromaLINK® Biotin Reagent) to NanoLINK beads.

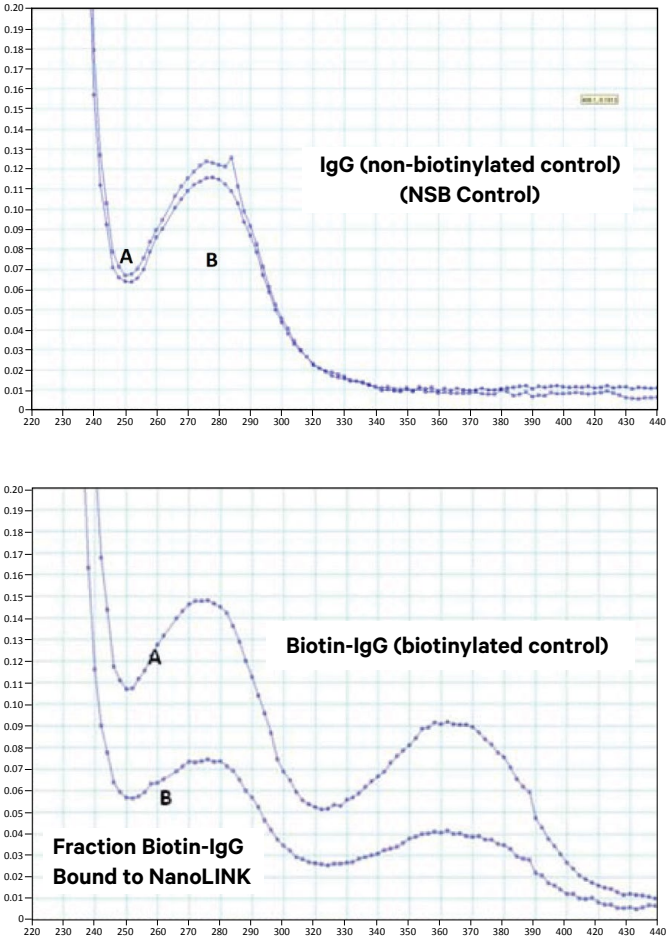

Figure 3 *Low background binding and high biotinylated-antibody capture efficiency.* ***Top graph:*** *The low NSB of biotinylated IgG to NanoLINK beads is demonstrated;* A: *UV scan of bovine IgG and* B: *supernatant of bovine IgG and NanoLINK streptavidin beads.* ***Lower graph:*** *Demonstration of biotin binding capacity of NanoLINK beads by addition of 250 µg biotinylated bovine IgG (3.4 biotins/bovine IgG as determined by use of ChromaLINK Biotin biotinylation reagent) to 0.5 mg of NanoLINK beads.*

Conclusion

NanoLINK Streptavidin Magnetic Beads offer unparalled binding capacity, allowing scientists to optimize the efficiency of their magnetic bead-based assays.

The following resources are available to successfully use NanoLINK Streptavidin Magnetic Beads and ChromaLINK Biotin Labeling Reagent:

NanoLINK Protocol
ChromaLINK Biotin Protocol

Product Selection Guide

Product	Size	Catalog No.
NanoLINK® Streptavidin Magnetic Beads	1 ml at 10 mg/ml	M-1002-010
ChromaLINK® Biotin Protein Labeling Kit	Kit – Five reactions of 25 µg to 1 mg	B-9007-105
ChromaLINK® One-Shot™ Antibody Biotinylation Kit	Kit – Labels 100 µg of Ab	B-9007-009
Sulfo ChromaLINK® Biotin (Water Soluble)	10 mg	B-1007-110

For custom bioconjugation services visit
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