How to Biotinylate with Quantifiable Results

Introduction

The Biotin-Streptavidin system continues to be used in many protein-based biological research applications including; ELISAs, immunoprecipitation, Westen blotting, general immobilization and detection, and many other biological procedures. Although pre-biotinylated proteins are often available from commercial sources, there are many instances when specialized proteins are not available in this form; thereby requiring the researcher to biotinylate their own protein.

However, there are many common problems and other pitfalls associated with standard biotinylation procedures, for example;

- A researcher is often uncertain a reaction worked properly or even to what degree
- Over-biotinylation often causes precipitation and loss of protein
- Over-biotinylation often reduces protein activity and/or function

For this reason, streptavidin-binding assays were developed and used to quantify the degree of protein biotinylation. For example, two such assays include the HABA and a FluoroReporter™ streptavidin binding assay. However, streptavidin binding assays suffer from numerous shortcomings:

- High cost (laborious and time-consuming)
- Require expensive, often unavailable equipment (e.g. fluorimeter)
- Require external protein calibration curve
- Binding assays are destructive in nature and consume significant quantities of often precious protein
- Binding assays almost always underrepresent the number of biotin molecules actually attached to the protein

How can these problems be solved?

1) ***Control the amount of biotin on your protein for assay optimization***

In order to avoid over modification which often causes precipitation and or greatly reduces activity you should select a directly traceable biotinylation kit. Such a kit would allow you to quickly determine the amount of biotin incorporated into a protein before every assay. Such traceable biotinylation would enable you to quickly determine the number of biotin molecules present on the protein and thus allow a minimal amount of biotin- just enough to successfully complete an assay without disrupting activity or function. A fast, reliable and easy to use method for determining the number of biotins attached to a protein would eliminate the desire to move on blindly to the next step of an often complicated downstream assay.

2) ***Biotin quantification without expensive equipment and additional costly assays.***

Many of the current methods for quantifying biotin on proteins require a secondary assay such as the HABA or the FluorReporter™ (Invitrogen) assay. The former assay is a destructive assay that is less sensitive and can consume up to 75 ug of the labeled protein in the assay. It also requires an external streptavidin-based calibration curve. The latter, FluoroReporter™ assay, although more sensitive than the HABA assay, requires a spectrofluorimeter or a fluorescent plate reader. This assay also requires an external calibration curve. The destructive nature of the assays along with increased labor cost and time diminish the general implementation of these assays. Any method for circumventing these limitations could be quite beneficial to any small company or research lab that worries about the cost of such ancillary reagents and assays.

3) ***Reproduce your results effectively.***

Quick and accurate quantification of the number of biotins incorporated in a protein will allow you to quantify your reactions each time you biotinylate. This provides confidence in the quality of the assay reagents being used. It also permits quantitative comparison to previous biotinylation reactions. Quantifying the biotin MSR (biotin molar substitution ratio) after labeling would allow you move on to the next step of a process or assay with much greater confidence.

4) ***Choose a traceable biotin reagent with a "built-in" signaling system.***
When you choose the right biotin reagent, you will ensure tracking and identification of the entire labeling process, always 'dialing in' and quantifying the proper degree of biotin incorporation before every assay or process.

<u>Current biotinylation products on the market that can help solve these problems:</u>

Although there are several products on the market to address one or two of the solutions above, SoluLink offers the only comprehensive solution to all these problems in a single reagent. We also provide easy to use automated calculators that avoid any need to manually calculate how much reagent to use or time consuming calculations. SoluLinK also offers one-on-one technical support for any biotinylation project and/or other conjugation support services. Solving all these problems with the use of a single reagent can help you **achieve your ultimate research goals** while saving you time, money, protein, and other valuable resources while providing valuable process information.

Control the amount of biotin on the protein

In order to address all of the common biotinylation problems, Solulink has developed ChromaLink Biotin **(Figure 1)**. ChromaLink Biotin is a water-soluble biotin labeling reagent with built-in signal traceability that allows you to track and rapidly calculate the exact number of biotins attached to a protein or antibody. The procedure for labeling with ChromaLink Biotin is identical to biotinylating with any other NHS-based biotinylation reagent. The key to solving the common problems previously discussed revolve around the unique, UV-traceable chromophore embedded

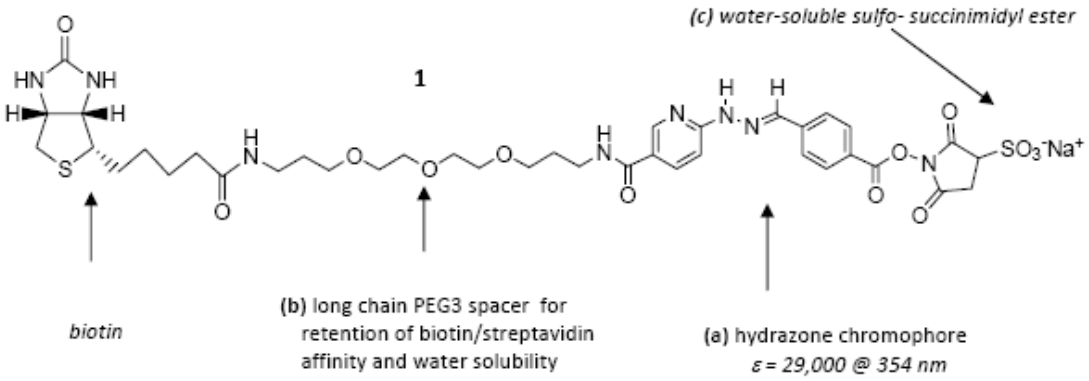

Figure 1. Structure and chemical composition of Sulfo-ChromaLink Biotin ($C_{38}H_{49}N_8NaO_{13}S_2$) (M.W. 912.96)

within the linker itself. Following buffer exchange of the labeling reaction, the biotinylated protein is simply analyzed by measuring the A280 and A354 of the conjugate. Inserting the absorbance values into a ChromaLink Biotin calculator (provided) automatically calculates the final protein concentration and the number of biotins incorporated!

Biotin quantification using a simple spectrophotometer and no other costly reagents

Representative UV absorbance spectra of a biotinylated antibody using ChromaLink Biotin can be used to illustrate how easy it is to quantify biotin incorporation by a simple scan of the biotinylated sample (Figure 2). Data can be acquired on any conventional or NanoDrop™ spectrophotometer and the sample recovered after analysis (non-destructive).

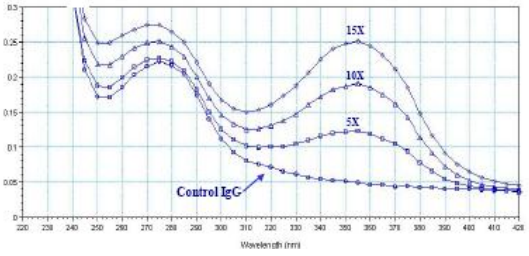

Figure 2: Overlaid UV absorbtion spectra of buffer exchanged bovine lgG biotinylated using ChromaLink™ Biotin at 5x, 10x and 15x equivalents of reagent over protein.

Avoid Additional Costly Assays

In order to illustrate how the HABA assay (Pierce Chemical Co., Rockford, IL) often under reports the number of incorporated biotins versus the ChromaLink Biotin method, both assays for biotin incorporation were compared and results summarized in **Table 1**. Data in the table was generated by biotinylating **(**500 ul @ 5 mg/ml) of a bovine IgG sample at 5, 10, and 15 mole equivalents using ChromaLink Biotin.

As seen from the results, HABA measurements yield lower estimates of biotin incorporation resulting in significant differences between the two assays. For example, the biotin molar substitution ratio calculated using the HABA dye-binding assay is generally 1⁄3 the value obtained with the ChromaLink method. The HABA dye-binding assay generally underestimates the true biotin molar substitution ratio because it measures the number of moles of biotin available for **binding to streptavidin** and not the absolute number of biotin molecules attached to the antibody surface. For example, two biotin molecules in close proximity to each other are likely to bind to a single streptavidin molecule.

Mole equivalents biotin reagent added	Biotin/IgG HABA	Biotin/IgG ChromaLink Biotin @ A354
5X	1.03	2.45
10X	1.60	4.71
15X	2.22	6.25

Table 1. Measured biotin molar substitution ratio (MSR) obtained using two different biotin incorporation methods on the same sample (ChromaLink™ UV-spectrophotometric assay @ 354 nm vs. the HABA assay).

Label Reproducibility

Triplicate biotinylation reactions were set-up using bovine IgG @ 5 mg/ml and 6 equivalents of ChromaLink Biotin reagent. After purification, each sample was scanned using a NanoDrop™ spectrophotometer (Figure 3), and the resultant spectra overlaid. As clearly demonstrated, results are easy to confirm and reproduce, time after time.

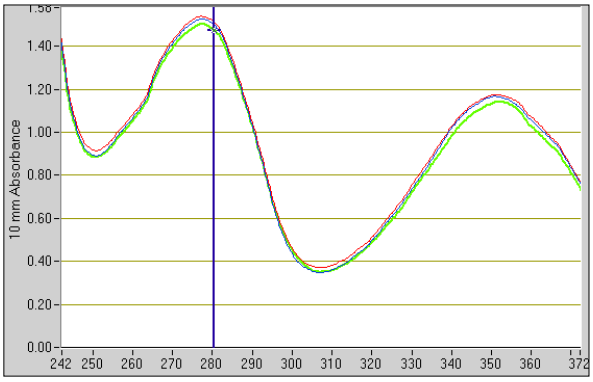

Figure 3. Overlaid spectra confirming reproducibility of antibody biotinylation (triplicates).

Make Assay Optimization Simple

Direct ELISA

A goat anti-bovine IgG antibody was biotinylated using ChromaLink™ Biotin to obtain a series of different molar substitution ratios. The biotinylated antibodies were then used to detect immobilized antigen (bovine IgG) in a standard ELISA procedure. Purified bovine IgG was immobilized (2-fold dilution series) (0.5 - 5,000 ng/ml). After immobilization (4 hr @ RT), wells were blocked with 1% casein/PBS and subsequently washed. The plate was then incubated with streptavidin-HRP @ 1 µg/ml for 60 minutes. After washes, TMB substrate (3,3',5,5'- tetramethylbenzidine) was added for 20 minutes. Signals were measured on a conventional plate reader @ 650 nm. Direct ELISA dose response curves were plotted as illustrated below

Results: *Signal/noise increased approximately 2.9-fold (linear portion of the curve) as the biotin MSR increased from 1.3 to 6.1 as illustrated in Figure 4. Background controls were constant across the various MSRs (data not shown).*

To further illustrate the relationship between signal/noise and MSR for this antibody/antigen pair, plots were generated at a single fixed antigen concentration (e.g.2 ng/well) across a range of molar substitution ratios (**Figure 5**).

Results*: Measured signal/noise increases almost 2.9-fold as the MSR goes from 1.3 to 6.1. Note the slight reduction in signal as the MSR goes beyond 6.1 probably due to over-modification of the antibody.*

Conclusion: You have more important things to do than worry about biotinylating a protein. ChromaLink Biotin allows you to biotinylate proteins quickly and easily and then confirm the number of biotins incorporated so you can proceed with confidence!!

Recommended Products:
[B-9007-105K] ChromaLink™ Biotin Labeling Kit
[B-9007-105K] ChromaLink™ One-Shot Kit
[B-1007-110] ChromaLink™ Labeling Reagent

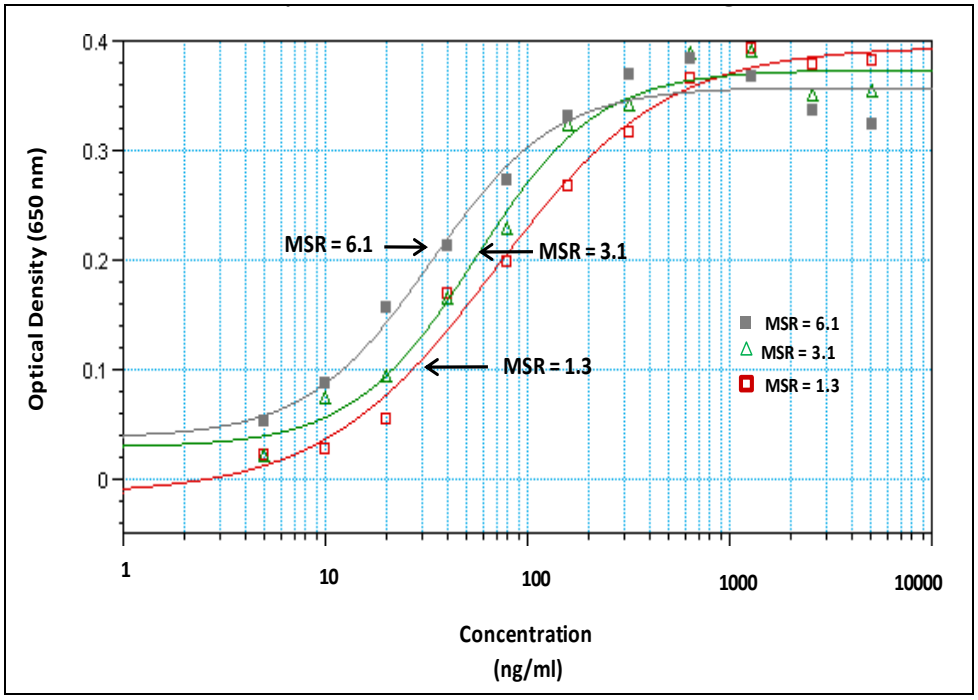

Figure 4. Direct ELISA response curves illustrating the relationship between biotin molar substitution ratio and direct ELISA signals @ 650 nm for an anti-bovine IgG biotin conjugate.

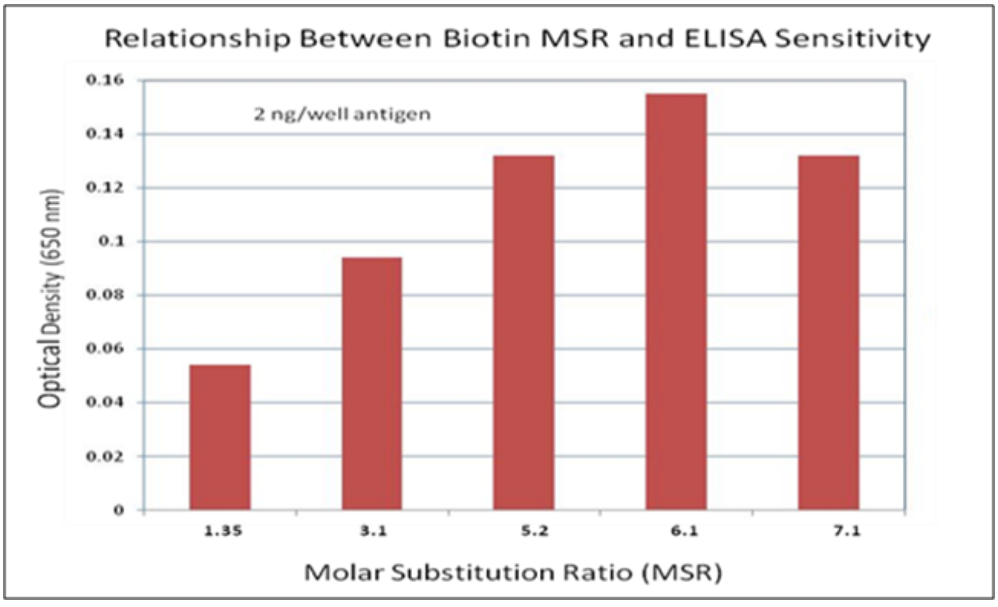

Figure 5. Background corrected direct ELISA signals at a fixed quantity of immobilized antigen (i.e. 2 ng per well) vs. MSR. Note the gradual increase in S/N (~ 2.9-fold) as the MSR increases from 1.3 to 6.1.