WHITE PAPER

How to Biotinylate with Reproducible Results

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Introduction

The biotin-streptavidin system continues to be used in many protein-based biological research applications including ELISA, immunoprecipitation, western blotting, general immobilization and detection, and many other experimental procedures. Although ready-made biotinylated proteins are often available from commercial sources, there are many instances when specialized proteins are not available in this form, requiring the researcher to biotinylate their own protein.

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

- Uncertainty about whether 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, biotin quantitation assays were developed and used to quantify the degree of protein biotinylation. For example, two such dye-based assays include the HABA and FluoReporter® biotin quantitation assay (Invitrogen™). However, such 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 attached to the protein

How can these problems be solved?

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

To avoid over-modification, which often causes precipitation and/or greatly reduces activity, you should select a directly traceable biotinylation kit. Such a kit will allow you to quickly determine the amount of biotin incorporated into a protein before every assay. Biotinylation traceability will enable quick determination of the number of biotin molecules present on the protein and thus allow use of the minimal amount of biotin 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 will eliminate the desire to move on to the next step of an often complicated downstream assay without knowing the biotinylation was successful.

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 FluoReporter assay. The former assay is a destructive assay that is less sensitive and can consume up to 75 µg of the labeled protein in the assay. It also requires an external streptavidin-based calibration curve. The latter FluoReporter 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 these 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 anyone with concerns 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 molar substitution ratio (biotin MSR) after labeling will allow you to 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 confirming every assay or process.

Current biotinylation products on the market that can help solve these problems.

Although there are several products on the market to address 1 or 2 of the problems above, there is just 1 comprehensive solution to address these issues. Also provided are easy-to-use automated calculators that avoid any need to manually calculate how much reagent to use or other time-consuming calculations. One-on-one technical support for any biotinylation and/or other conjugation project using SoluLINK® technology is also available. 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 and providing valuable process information.

Control the amount of biotin on the protein.

To address all of the common biotinylation problems, you can rely on ChromaLINK® Biotin (Figure 1), a water-soluble biotin labeling reagent with built-in signal traceability to enable you to 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 within the linker itself. Following buffer exchange of the labeling reaction, the biotinylated protein is simply analyzed by measuring the A_{280} and A_{354} of the conjugate. Inserting the absorbance values into the provided ChromaLINK Biotin calculator automatically calculates the final protein concentration and the number of biotins incorporated.

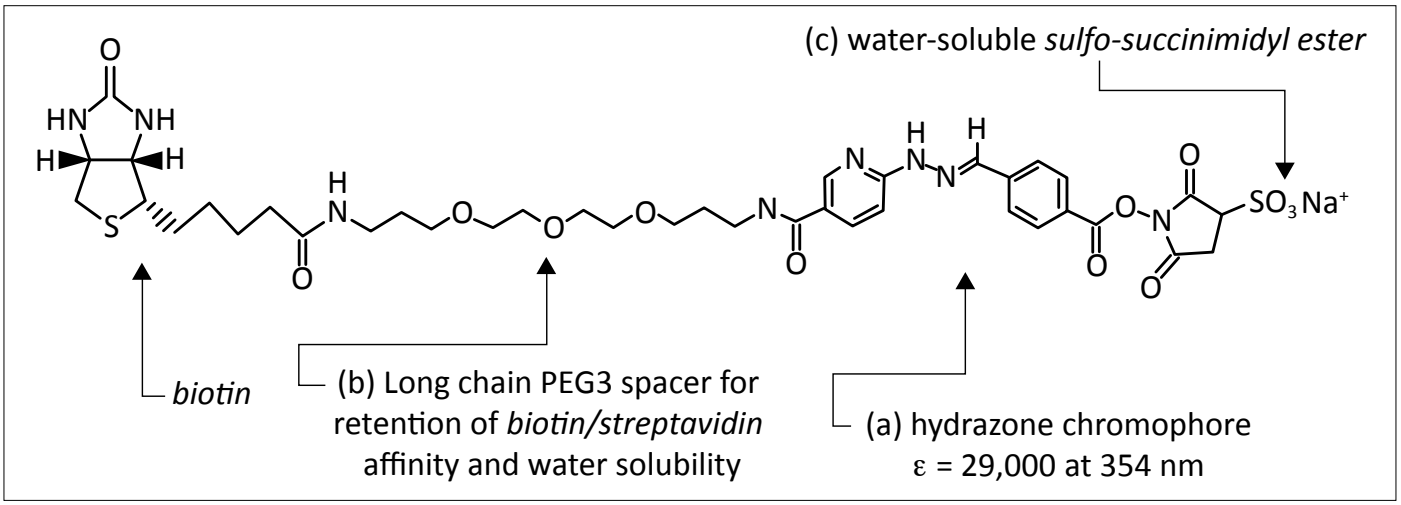

Figure 1 *Structure and chemical composition of Sulfo-ChromaLINK Biotin ($C_{38}H_{49}N_8NaO_{13}S_2$) (MWt 912.96).*

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 can be recovered after analysis (non-destructive).

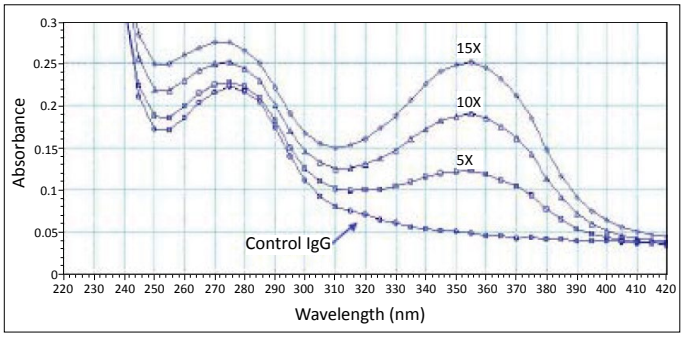

Figure 2 *Overlaid UV absorption spectra of buffer exchanged bovine IgG biotinylated using ChromaLink Biotin at 5x, 10x, and 15x equivalents of reagent over protein.*

Avoid Additional Costly Assays

To illustrate how the HABA assay often underreports 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 µl at 5 mg/ml) a bovine IgG sample at 5, 10, and 15 mole equivalents using ChromaLINK Biotin.

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

Table 1 *Measured biotin MSR obtained using two different biotin incorporation methods on the same sample (ChromaLINK UV-spectrophotometric assay at 354 nm vs. the HABA assay.)*

As seen from the results, HABA measurements yield lower estimates of biotin incorporation, presenting as significant differences between the two assays. For example, the biotin molar substitution ratio calculated using the HABA dye-binding assay is generally one-third the value obtained with the ChromaLINK method. The HABA dye-binding assay generally underestimates the true biotin MSR 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.

Label Reproducibility

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

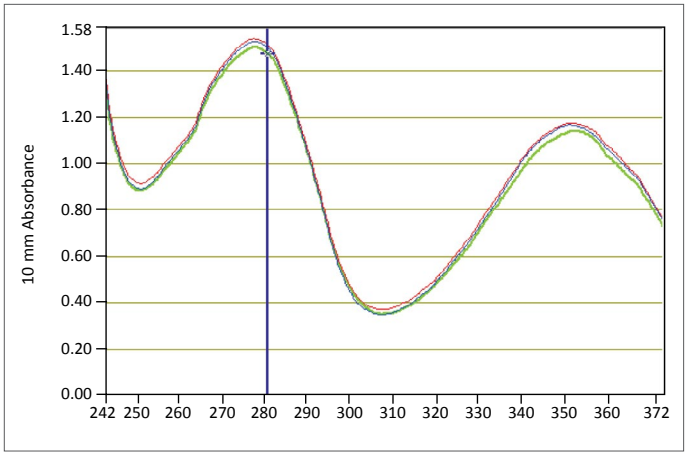

Figure 3 *Overlaid spectra confirming reproducibility of antibody biotinylation (triplicates).*

Make Assay Optimization Simple

Direct ELISA

A goat anti-bovine IgG antibody was biotinylated using ChromaLINK Biotin to obtain a series of different biotin MSRs. 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 h at RT), wells were blocked with 1% casein/PBS and subsequently washed. The plate was then incubated with streptavidin-HRP at 1 µg/ml for 60 min. After washes, TMB substrate (3,3',5,5'- tetramethylbenzidine) was added for 20 min. Signals were measured on a conventional plate reader at 650 nm. Direct ELISA dose response curves were plotted in Figure 4.

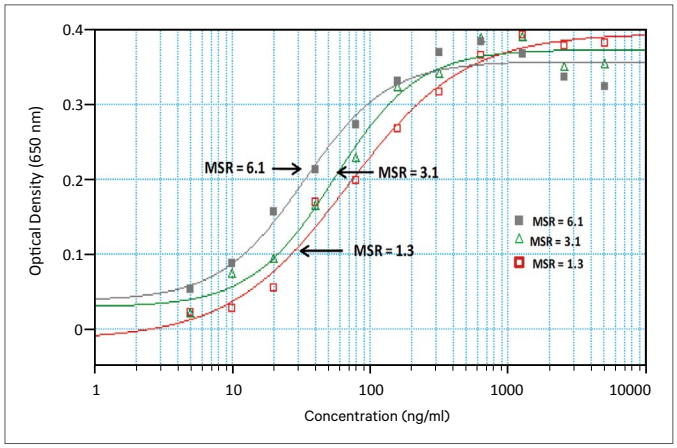

Figure 4 *Direct ELISA response curves illustrating the relationship between biotin MSR and direct ELISA signals at 650 nm for an anti-bovine IgG biotin conjugate.*

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

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-9007-105]** ChromaLINK® Biotin ProteinLabeling Kit
- **[B-9007-009]** ChromaLINK® One-Shot™ Antibody Biotinylation Kit
- **[B-1007]** Sulfo ChromaLINK® Biotin (Water Soluble)

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

Results: *Measured signal-to-noise increases almost 2.9-fold as the MSR goes from 1.35 to 6.1. Note the slight reduction in signal as the MSR goes beyond 6.1, probably due to over-modification of the antibody.*

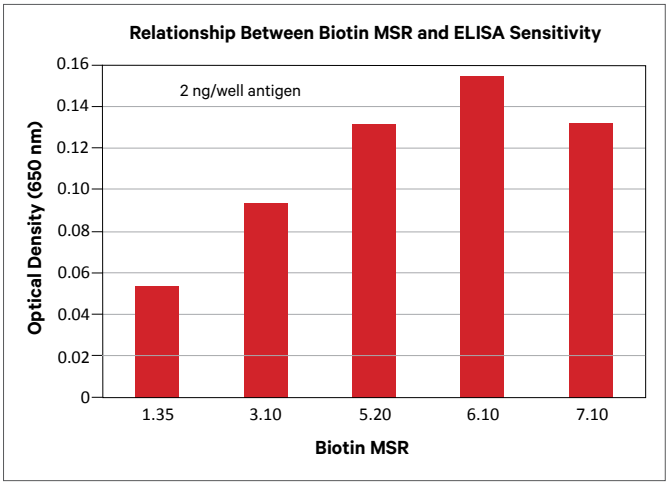

Figure 5 *Background-corrected direct ELISA signals at a fixed quantity of immobilized antigen (i.e., 2 ng per well) vs. biotin MSR. Note the gradual increase in signal-to-noise (~2.9-fold) as the biotin MSR increases from 1.35 to 6.10.*

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