



## SureLINK™ Chromophoric Biotin Labeling Kits

### Stalking the elusive easy-to-use kit for quantitative protein labeling?

SureLINK™ Chromophoric Biotin Labeling Kits provide an easy-to-use method for biotinylating antibodies and other proteins. These kits label proteins with biotin and enable easy measurement of biotin incorporation with just one reagent.

Accurate measurement of the number of biotins attached to a protein is important in controlling the reproducibility of biotin conjugates and has a direct impact on assay reproducibility. SureLINK Chromophoric Biotin Labeling Kits provide a quick and reliable method for estimating the amount of biotin in the biotin-protein conjugate. In contrast, the standard HABA (4'-hydroxyazobenzene-2-carboxylic acid) method for measuring incorporated biotin is inaccurate, lengthy and tedious.

#### More accurate quantitation than HABA

Optimize your biotin conjugates the first time! With SureLINK (Figure 1) a simple spectrophotometric analysis at 354 nm enables quantitation of incorporated biotin. It is more accurate than the HABA reagent which binds to avidin and absorbs at 500 nm. Biotin competes with HABA for binding to avidin. The resulting absorbance change is small and difficult to measure reproducibly.

#### Convenient and fast

Get results in minutes! SureLINK Chromophoric Biotin labels your protein and measures the extent of labeling using a quick protocol. Biotin is provided in convenient reaction size vials, no weighing required.

#### Save precious protein sample

HABA consumes precious biotinylated sample and can only be used with high concentrations of protein. Unlike HABA, SureLINK accurately measures biotin incorporation even at low concentrations of protein, and does not consume biotinylated protein in the process.

#### Improve conjugate performance



**Figure 1: Structure of SureLINK Chromophoric Biotin**  
Biotin (blue); PEG spacer (red); bis-aryl hydrazone chromophore (green); Amine-reactive succinimide ester (black)

Improve detection in your immunoassay! SureLINK Chromophoric Biotin contains a long spacer arm (Figure 1) which improves solubility of the conjugate and minimizes steric hindrance among multiple biotinylated sites in detection applications. It also has an amine-reactive succinimide ester (NHS ester) that efficiently couples to protein primary amine groups.

No need to look further You've just found the conjugation kits that deliver outstanding assay results. Try SureLINK and SEE MORE with KPL!

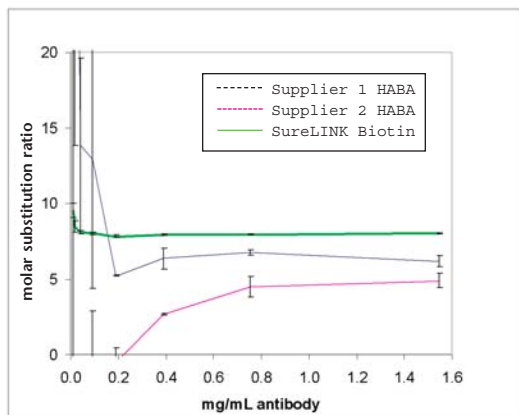


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## SureLINK Chromophoric Biotin Labeling Kits

### SureLINK Biotin assures accurate quantitation at low protein concentrations.

A comparison of the reproducibility of biotin quantitation using SureLINK and the traditional HABA method reveals the limitations of HABA (Figure 2). Commercially available HABA kits from two different suppliers were tested. The change in absorbance due to addition of biotinylated antibody was very small relative to the baseline  $A_{500}$ . These small decreases in absorbance are difficult to measure reproducibly. The results indicate the variability of HABA, especially at low protein concentrations (<0.5 mg/mL). By contrast, the biotin conjugate prepared with SureLINK has an absorbance at 354 nm, well within the linear range of the spectrophotometer, even at low (<0.1 mg/mL) protein concentrations.



**Figure 2:** A single biotin conjugate of mouse monoclonal antibody was prepared using the SureLINK Chromophoric Biotin Labeling Kit. The conjugate was serially diluted and biotin content tested using commercially available HABA assay kits from two different manufacturers and by direct measurement of the chromophoric biotin at  $A_{354}$ .

## Ordering Information

SureLINK Chromophoric Biotin Labeling Kits contain SureLINK Chromophoric Biotin, 10X Modification Buffer, Anhydrous DMF, and Spin Filters (5K MWCO).

Catalog#	Description	Size
86-00-01	SureLINK Chromophoric Biotin Labeling Kit Each 1.0 mg vial of biotin is sufficient for labeling approximately 3 - 20 mg protein.	5 x 1 mg biotin
86-00-02	SureLINK Chromophoric Biotin Labeling Kit Each 0.5 mg vial of biotin is sufficient for labeling approximately 1.5 - 10 mg protein.	2 x 0.5 mg biotin
86-00-03	SureLINK Chromophoric Biotin Labels approximately 30 - 200 mg protein	10 mg
60-00-53	Spin-Pure Filters, 10K MWCO	

To order or for more information, contact us at 800.638.3167 / 301.948.7755, fax 301.948.0169 or visit us at [www.kpl.com](http://www.kpl.com).

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*Jaguar cub*  
Better conjugates make better immunoassays. SEE MORE with KPL!

## SureLINK™ Chromophoric Biotin Labeling Kits

### Frequently Asked Questions

*What chemistry is used in the SureLINK Chromophoric Biotin Labeling Kits?*

SureLINK Chromophoric Biotin Labeling Kits are based on the well-established N-hydroxysuccinimide (NHS) chemistry. The Chromophoric Biotin contains a reactive NHS ester group that readily couples with primary amines present on proteins at pH 7.0-9.0 to form an amide bond. The conjugation reaction can be carried out using incubation times from 2 hours to overnight.

*What biomolecules can be conjugated with the SureLINK Chromophoric Biotin Labeling Kits?*

Proteins, peptides and oligonucleotides can be conjugated using this technology. Proteins and peptides can be biotinylated using free amine groups on lysine side chains and the N terminus. Modified oligonucleotides with primary amine groups are also suitable for biotinylation.

*What determines the degree of biotin incorporation?*

The degree of biotin incorporation depends on the concentration of protein, the molar excess of biotin added, the availability of primary amines in protein, and the incubation temperature and time of conjugation reaction.

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