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## KPL SureLINK™ Chromophoric Biotin Manual

Products	Catalog No.
KPL SureLINK™ Chromophoric Biotin Labeling Kit, 5 x 1 mg	5610-0027 (86-00-01)
KPL SureLINK™ Chromophoric Biotin, 10 mg	5620-0030 (86-00-03)





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## PRODUCT DESCRIPTION

KPL SureLINK™ Chromophoric Biotin Labeling Kit provides all the reagents needed to label antibodies, proteins and other macromolecules with biotin through the modification of primary amines. This product contains an activated form of biotin that contains a unique chromophore in the linker region. The kit also contains organic solvent, modification buffer, and spin filters for purifying the biotin conjugates.

### BACKGROUND

Biotin binds to streptavidin/avidin conjugates with high affinity. Antibodies or protein conjugated with several biotin molecules can amplify signal through streptavidin-conjugated reporter enzyme, thereby increasing the sensitivity of immunoassays. Biotinylated antibodies or proteins can be used in a variety of applications including ELISA, blotting, and immunohistochemistry applications.

It is important to know the extent of biotin modification for effective assay development. While there are many reagents available for biotinylating proteins, determining the amount of biotin incorporated has historically depended on the use of the 4'-hydroxyazobenzene-2-carboxylic acid (HABA) reagent. HABA binds weakly to avidin and absorbs at 500 nm. Biotin competes with HABA for binding to avidin, thereby lowering the absorbance at 500 nm. This method consumes precious sample and is only useful for concentrated proteins because the difference in absorbance due to biotin displacement of HABA from avidin is very small and difficult to accurately measure at low concentrations.

The KPL SureLINK™ Chromophoric Biotin used in these kits contains a *bis*-aryl hydrazone chromophore (Figure 1-3), whose absorptivity ( $\epsilon = 29,000 \text{ M}^{-1} \text{ cm}^{-1}$  at 354 nm) allows easy, accurate and nondestructive quantitation of biotin incorporation, even at low ( $<0.1 \text{ mg/ml}$ ) protein concentrations. The reagent also contains a biotin moiety (Fig. 1-1), a long chain PEG spacer (Fig. 1-2) for improved aqueous solubility, and an aromatic *N*-hydroxysuccinimide (NHS) ester leaving group (Fig. 1-4) that reacts efficiently with primary amino groups ( $-\text{NH}_2$ ) in pH 7-9 buffers (i.e. protein lysine residues and the N-terminal amino group) to form stable amide bonds.

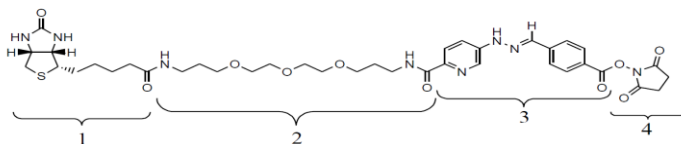


Figure 1. Chemical structure of KPL SureLINK™ Chromophoric Biotin

## PRODUCT COMPONENTS

### 5610-0027 (86-00-01), KPL SureLINK™ Chromophoric Biotin Labeling Kit

Size: 5 x 1 mg

Kit Component	Part Number	Size	Quantity
KPL SureLINK™ Chromophoric Biotin	5620-0031 (86-01-02)	1 mg	5
KPL Modification Buffer	5640-0007 (82-01-02)	5 mL	2
KPL Anhydrous DMF*	5640-0008 (82-01-03)	1.5 mL	1
KPL Spin-Pure Filters (10K MWCO)**	5640-0001 (60-00-53)	N/A	5

### 5620-0030 (86-00-03), KPL SureLINK™ Chromophoric Biotin

Size: 10 mg

Kit Component	Part Number	Size	Quantity
KPL SureLINK™ Chromophoric Biotin	5620-0030 (86-00-03)	10 mg	1

Kit utilizes convenient single use biotin vials to avoid the need to weigh out chemicals and to preserve chemical stability.

- 5 x 1 mg kit 5610-0027 (86-00-01): 5 individual vials of biotin, each contains 1 mg biotin for labeling up to 3-20 mg antibody or other proteins.
- 10 mg biotin 5620-0030 (86-00-03): sufficient for labeling 30-200 mg antibody or other proteins.

\* Anhydrous DMF contains molecular sieves to maintain anhydrous conditions. \* Anhydrous DMF contains molecular sieves to maintain anhydrous conditions. FITC and FAM-X vials are packed in resealable foil bags with desiccants.

\*\* Additional spin filters can be purchased 5640-0001 (60-00-53). The other components in the kits are not sold separately.

## STORAGE AND STABILITY

- ◆ KPL SureLINK™ Chromophoric Biotin Labeling kit is shipped at ambient temperature.
- ◆ Store all components at 2-8°C upon receipt and store KPL SureLINK™ Chromophoric Biotin under desiccation.
- ◆ All components of KPL SureLINK™ Chromophoric Biotin Labeling kit are stable for at least one year when stored at recommended conditions.

## BEFORE YOU BEGIN

### SAFETY AND HANDLING

- ◆ Read MSDS and all instructions thoroughly before using kits.
- ◆ Wear appropriate personal protective equipment when handling reagents.

### OTHER REQUIRED SUPPLIES AND EQUIPMENT

- ◆ Antibody or protein, free of salts or contaminants. See Troubleshooting section for removing salts or other contaminants. Additional KPL Modification Buffer (100 mM phosphate, 150 mM NaCl, pH 7.2-7.4) may be required for buffer exchange.
- ◆ Molecular biology grade water
- ◆ Shaker capable of gentle agitation
- ◆ Vortex
- ◆ Microcentrifuge

### REAGENT PREPARATION

- ◆ Dissolve KPL SureLINK™ Chromophoric Biotin in DMF immediately prior to use. Tap down and equilibrate the biotin vial to room temperature prior to opening to avoid moisture condensation. The NHS-ester moiety of KPL SureLINK chromophoric biotin is hydrolyzed when exposed to water.

Extended storage of the biotin/DMF solution is not recommended. Aliquots may be stored at -20°C under desiccation for short periods of time.

## QUICK REFERENCE PROTOCOL

Rehydrate or transfer antibody/protein in an appropriate buffer



Dissolve KPL SureLINK™ Chromophoric Biotin in DMF at  
20 mg/mL



Add biotin to protein solution



Incubate the reaction mixture at room temperature for 2 hours  
with gentle agitation



Remove unconjugated biotin from protein using spin filter or by  
dialysis



Measure degree of biotin incorporation with spectrophotometer

<p>Elapsed time: approximately 3 hr Hands-on time: approximately 30 min</p>
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## BIOTIN CONJUGATION PROTOCOL

This protocol describes the conjugation reaction for **1 mL of IgG at 0.2 to 5.0 mg/mL**. Other volumes and concentrations can be used (see Calculations section). Other proteins can also be labeled using this protocol.

1. **Rehydrate or transfer the antibody into 1 mL Modification Buffer (100 mM phosphate, 150 mM NaCl, pH 7.2-7.4) at 0.2-5.0 mg/mL.**

Buffers containing Tris, imidazole, glycine or primary amines should not be used due to competition with the conjugation reaction. If the sample is stored in one of these buffers, remove by buffer exchange against the Modification Buffer. PBS (10 mM phosphate, 150 mM NaCl, pH 7.2-7.4) is not recommended due to poor buffering capacity.

2. **Immediately prior to use, prepare a 20 mg/mL (25 nmole/ $\mu$ L) stock solution of SureLINK™ Chromophoric Biotin in anhydrous DMF.**

KPL SureLINK™ Chromophoric Biotin	DMF to prepare a 20 mg/mL of chromophoric biotin
1.0 mg	50 $\mu$ L
10.0 mg	Variable, depending on the weighed amount

Tap down and equilibrate biotin vial to room temperature prior to opening to avoid the condensation of moisture. The NHS-ester moiety of chromophoric biotin is hydrolyzed when exposed to water.

If high biotin molar excess is chosen for a highly concentrated protein solution, the concentration of biotin stock solution can be increased to 40 mg/mL (see Calculation section for more details).

3. **Add an appropriate volume of 20 mg/mL KPL SureLINK™ Chromophoric Biotin to the antibody or protein solution.** See the Calculation section Part 2 to determine the appropriate volume of KPL SureLINK™ Biotin. The solution may become cloudy, which does not affect the labeling reaction. The percentage of biotin solution added should be less than 5% of



the total reaction volume to minimize protein precipitation by DMF (Melnikova et al.).

**Examples for Conjugation Reactions:** When antibodies at 1 mg/mL are used for labeling, a 40-fold molar excess of biotin is recommended for ELISA and a 10-fold molar excess of biotin is recommended for Western blotting.

Volume of IgG solution (µL)	Protein conc. (mg/mL)	Total IgG amount (mg)	Biotin molar excess (fold)	Volume of 20 mg/mL biotin stock (µL)	DMF % in reaction mixture
1000	0.2	0.2	60X	3.2	0.3
1000	0.5	0.5	60X	8.0	0.8
1000	0.5	0.5	40X	5.3	0.5
1000	1	1.0	40X	10.7	1.1
1000	1	1.0	10X	2.7	0.3
1000	4	4.0	40X	42.7	4.3
1000	4	4.0	10X	10.7	1.1

4. **Incubate at room temperature for two hours with gentle agitation.** Longer incubation times, up to overnight, may be used. However, microbial growth and protein degradation may be a concern.
5. **Remove the unconjugated chromophoric biotin by spin filter.** SeraCare recommends the following desalting procedure using the spin filters included in the kits:
  - a. Add the biotinylated protein solution to the top section of the spin filter. Each spin filter can hold up to 500 µL. Concentrate the sample by centrifugation at 12,000 x g for 30 minutes. The concentrated solution will remain in the top section of the spin filter. Dispose of the filtrate (bottom section) of the spin filter.
  - b. Repeat as necessary until a buffer exchange of at least 200-fold is achieved. Example – if 10 µL of sample remain in the top section, desalt the remaining sample with four 500 µL aliquots of buffer.

Desalting columns or dialysis cassettes can also be used to remove unconjugated biotin. Choose an appropriate molecular weight cutoff (MWCO) for your protein.

6. **The biotinylated protein is now ready to use. To determine the molar substitution ratio (MSR) of biotin to protein see the DETERMINING DEGREE OF BIOTIN INCORPORATION section.**

Determine the degree of biotin incorporation prior to adding any stabilizers to the biotinylated sample, such as BSA.

## **DETERMINING THE DEGREE OF BIOTIN INCORPORATION**

1. **Transfer the labeled protein sample into a cuvette and measure the absorbance at both 280 and 354 nm using a UV spectrophotometer. Both absorption values should fall within the linear range of the spectrophotometer, typically 0.05 – 2.00. If necessary, dilute the sample. Record the  $A_{280\text{nm}}$ ,  $A_{354\text{nm}}$ , and dilution factor (DF).**

The unconjugated chromophoric biotin must be removed from the biotinylated protein in order to determine the degree of biotin incorporation. Measuring the absorption of the biotinylated protein will not harm the sample, allowing 100% recovery of the sample. The  $A_{280\text{nm}}$  is used to calculate the amount of protein in the sample.

2. **If you do not know the molar extinction coefficient of the protein at 280 nm, measure the protein concentration by an alternative method, such as the BCA or Bradford Assay. These assays will consume some of your sample and are not necessary for IgG conjugates.**

For IgG, the molar extinction coefficient at 280 nm is  $210,000 \text{ M}^{-1} \text{ cm}^{-1}$ . The molar extinction coefficient can also be calculated as follows:

$$\varepsilon = E_{280\text{nm}}^{0.1\%} \times \text{MW} = E_{280\text{nm}}^{1\text{mg/mL}} \times \text{MW} = 0.1 \times E_{280\text{nm}}^{1\%} \times \text{MW}$$

**Example:** For IgG,  $E_{280\text{nm}}^{0.1\%} = 1.4$  and  $\text{MW} = 150,000 \text{ g/mol}$

$$\varepsilon = E_{280\text{nm}}^{0.1\%} \times \text{MW} = 1.4 \times 150,000 = 210,000 \text{ M}^{-1} \text{ cm}^{-1}$$

### 3. Calculate the degree of biotin incorporation/molar substitution ratio (MSR)

Abbreviations:

- $A_{\text{nm}}$  Absorbance at the given wavelength in nm
- DF Dilution factor
- $\epsilon$  Protein molar extinction coefficient ( $\text{M}^{-1}\text{cm}^{-1}$ ) at 280 nm
- $E_{280\text{nm}}^{0.1\%}$  Absorbance of 1 mg/mL protein at 280 nm,  $E_{280\text{nm}}^{\frac{1\text{mg/mL}}$
- $\ell$  Path length in cm (usually 1 cm for a cuvette)
- MW Protein molecular weight (g/mol)

**In the following example,  $A_{280\text{nm}} = 1.209$ ,  $A_{354\text{nm}} = 1.160$ , and  $\text{DF} = 1$**

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#### A. Molar concentration of biotin

KPL SureLINK™ Chromophoric Biotin contains a *bis*-aryl hydrazone with a molar extinction coefficient of  $29,000 \text{ M}^{-1}\text{cm}^{-1}$  at 354 nm. The molar concentration of biotin in the labeled protein is calculated as follows:

$$[\text{Biotin}] = \frac{A_{354\text{nm}} \times \text{DF}}{29,000 \times \ell}$$

$$\text{Example: } \frac{1.160 \times 1}{29,000 \times 1} = 4.0 \times 10^{-5} \text{ M Biotin}$$

#### B. Molar concentration of protein

The concentration of the biotinylated protein is easily determined from the  $A_{280\text{nm}}$  and molecular weight if the molar extinction coefficient of the protein is known. KPL SureLINK™ Chromophoric Biotin contributes to the absorbance of the protein at 280 nm, so a correction must be made to the  $A_{280\text{nm}}$ . Alternatively, the protein concentration can be calculated using total protein assays such as BCA (Pierce) or Bradford (Bio-Rad).

- Given the  $A_{280\text{nm}}$ ,  $A_{354\text{nm}}$ , and  $\epsilon$

For IgG,  $\epsilon = 210,000 \text{ M}^{-1}\text{cm}^{-1}$  at 280 nm

Note:

$$\epsilon = E_{280\text{nm}}^{0.1\%} \times \text{MW} = E_{280\text{nm}}^{1\text{mg/mL}} \times \text{MW} = 0.1 \times E_{280\text{nm}}^{1\%} \times \text{MW}$$

$$[\text{Protein}] = \frac{[A_{280\text{nm}} - (A_{354\text{nm}} \times 0.29)] \times \text{DF}}{\epsilon \times \ell}$$

**Example:** 
$$\frac{[1.209 - (1.160 \times 0.29)] \times 1}{210,000 \times 1} = \frac{0.8726}{210,000} = 4.155 \times 10^{-6} \text{ M IgG}$$

- Given the MW and concentration of the biotinylated protein (mg/mL) from an alternative method, such as the BCA or Bradford Assay

For IgG, MW = 150,000 g/mol

$$[\text{Protein}] = \frac{\text{protein concentration in mg/mL}}{\text{MW}}$$

**Example:** 
$$\frac{0.6233 \text{ mg/mL IgG}}{150,000 \text{ g/mol}} = 4.155 \times 10^{-6} \text{ M IgG}$$

### C. Degree of biotin incorporation/ molar substitution ratio (MSR)

The degree of biotin incorporation is given as a ratio of the molar concentration of biotin to the molar concentration of protein. This dimensionless number is termed the molar substitution ratio, abbreviated MSR.

$$\text{MSR} = \frac{[\text{Biotin}]}{[\text{Protein}]}$$

**Example:** 
$$\frac{4.0 \times 10^{-5} \text{ M Biotin}}{4.155 \times 10^{-6} \text{ M IgG}} = \frac{40}{4.155} = 9.63$$

## CALCULATIONS

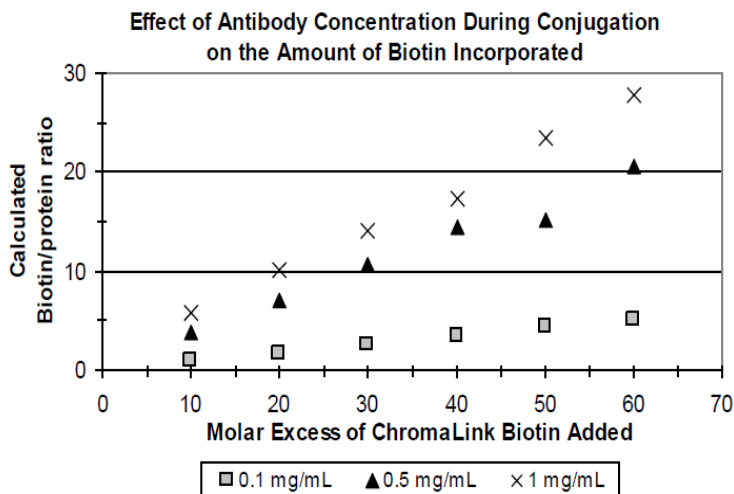
### Part 1: Determining the desired biotin:antibody (or protein) molar ratio for the conjugation reaction.

When labeling antibodies at a concentration of 1 mg/mL, SeraCare recommends a 40-fold molar excess of biotin for conjugates that will be used in ELISA and a 10-fold molar excess of biotin for conjugates that will be used in Western blotting. See “Use of biotin conjugates in various assays” section for more information.

Various biotin:antibody molar ratios can be used with the KPL SureLINK™ kit. In general, the degree of biotin incorporated depends on the following:

1. The concentration of protein to be biotinylated (Figure 2). Higher protein concentrations (>0.5 mg/mL) allow for the incorporation of biotin at higher rates.
2. The molar excess of biotin added. As seen in Figure 2, higher molar excesses of biotin improve incorporation.
3. The availability of primary amines.
4. The incubation time and temperature of the conjugation reaction.

**Figure 2. Biotin incorporation ratio with IgG:** The biotin incorporation ratio was determined empirically under different conditions. IgG at concentrations of 0.1 mg/mL, 0.5 mg/mL and 1 mg/mL were conjugated to biotin at a molar excess of 10-fold to 60-fold.



## Part 2: Determining the amount of KPL SureLINK™ Chromophoric Biotin for the conjugation reaction.

The volume of chromophoric biotin required for the conjugation reaction can be determined once the desired biotin molar excess ratio (See Part 1.) has been determined.

$$\text{Volume } (\mu\text{L}) \text{ of } 20 \text{ mg/mL biotin for conjugation reaction} = \frac{1000 \times (\text{protein amount in mg}) \times (\text{biotin molar excess ratio})}{(\text{protein molecular weight in KDa}) \times (25 \text{ nmole}/\mu\text{L})}$$

**Example:** 1 mg of IgG (MW 150 KDa) is biotinylated using a 40-fold biotin molar excess.

$$\text{Volume of } 20 \text{ mg/mL biotin} = (1000 \times 1 \times 40) / (150 \times 25) = 10.7 \mu\text{L}$$

## Part 3. Calculating the percentage of DMF in the conjugation reaction.

DMF exposure may cause irreversible conformational changes to antibodies and other proteins. These conformational changes may have impact on long term stability and antigen-binding activity. It is preferred to limit the percentage of DMF in conjugation reactions to less than 5% (Melnikova et al.).

If a large biotin molar excess is chosen for a highly concentrated protein solution, the DMF percentages may exceed 5% when a 20 mg/mL biotin stock solution is used. In these situations, the concentration of biotin stock solution can be increased to 40 mg/mL (50 nmol/ $\mu\text{L}$ ) to reduce the concentration of DMF.

Calculate the percentage of DMF using the following equation:

$$\% \text{ DMF} = \text{Vol. of biotin stock} \div \text{Vol. of protein to be biotinylated}$$

**Example:** using the example in Part 2, 10.7  $\mu\text{L}$  of 20 mg/mL biotin stock is added to 1000  $\mu\text{L}$  of 1 mg/mL IgG.

$$\% \text{ DMF} = 10.7 \mu\text{L} \div 1000 \mu\text{L} = 1.1\% \text{ DMF}$$

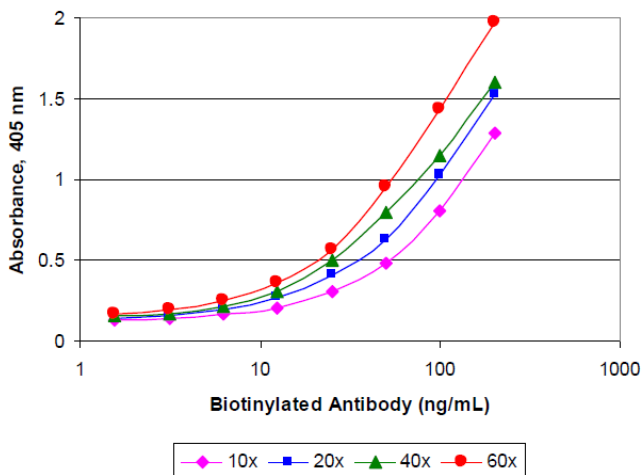
## RECOMMENDED USE OF CONJUGATES

KPL SureLINK™ Chromophoric Biotin Labeling kit can be used to label IgG or other proteins for a variety of immunoassay methods. The conjugate concentration that will provide the best signal to background in your specific assay may vary and should be determined for each conjugate. Include positive and negative controls in each immunoassay for proper review of experimental results and successful troubleshooting.

### Use of biotin conjugates in various assays.

The optimal biotin:protein ratio used in conjugation reactions can depend on the application. The number of biotins attached to a protein affects the sensitivity and reproducibility of immunoassays. KPL tested SureLINK™ chromophoric biotin antibody conjugates in ELISA, Western blotting and IHC. Antibodies at a 1 mg/mL concentration were conjugated with 10, 20, 40 and 60-fold excess biotin. ELISA sensitivity increased as the biotin conjugates were prepared with higher molar excess of biotin (Figure 3.).

Western blot sensitivity (not shown) was unaffected by conjugates prepared using higher molar excesses of biotin.



**Figure 3.** Detection of IgG in ELISA with varying levels of biotinylated antibody. KPL SureLINK™ Chromophoric Biotin conjugates of a 1 mg/mL antibody were made with 10, 20, 40, and 60-fold molar excess biotin. KPL Peroxidase-labeled streptavidin and KPL ABTS Substrate was used for detection at 405 nm.

For appropriate biotin conjugation concentrations to be used in ELISA, Western Blot, or immunohistochemistry, please refer to the Application Note for the KPL SureLINK™ Chromophoric Biotin Labeling kits or contact Technical Services.

## FREQUENTLY ASKED QUESTIONS

### ***Q: What biomolecules can be conjugated with the KPL SureLINK Chromophoric Biotin Labeling Kit?***

A: Proteins, peptides, and oligonucleotides can be conjugated using this technology. Proteins and peptides can be modified using primary amino groups on lysine residues and the N-terminus. Modified oligonucleotides with primary amino groups are suitable for conjugation.

### ***Q: What determine the degree of biotin incorporation?***

A: 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.

### ***Q: Is free chromophoric biotin a concern following the conjugation protocol?***

A: In immunoassays such as ELISA and Western blotting, the effect of free chromophoric biotin is minimal as most of the unconjugated biotin is removed in the washing steps. However, free biotin must be removed in order to quantitate the amount of biotin incorporation.

### ***Q: How do I know how many primary amines my protein has?***

A: Without knowledge of the protein sequence, the number of primary amines can be estimated by multiplying the MW of the protein by 0.0006. This formula estimates the number of lysine residues given an average weight of 110 Daltons for an amino acid, and an average lysine content of 6.6% in a protein (Dayhoff, M.O., 1978). Due to steric or functional constraints, some lysine residues may not be available for conjugation.

### ***Q: How much biotin should I use in the conjugation reaction?***

A: In general, adding between 10 and 60-fold molar excess of KPL SureLINK™ Chromophoric Biotin is sufficient for most applications. The optimal amount of biotin added will depend on the number of available primary amines, the assay conditions, the protein concentration, the incubation temperature, and the incubation time. A two hour, room temperature antibody conjugation reaction at a 5 mg/mL concentration with 40-fold molar excess of biotin works well in ELISA. We recommend



preparing multiple conjugates with various molar ratios of biotin. Over-modification of the protein with biotin may inhibit protein function.

***Q: Unlabeled protein samples are stored in buffer with Tris, sodium azide, or glycine. Can I still conjugate the proteins to KPL SureLINK Chromophoric Biotin?***

A: Tris, sodium azide and glycine will react with the KPL SureLINK Chromophoric Biotin in the conjugation reaction. Remove them by buffer exchange against 0.1 M sodium phosphate, 0.15 M NaCl, pH 7.2-7.4.

***Q: Do I have to dissolve KPL SureLINK™ Chromophoric Biotin in DMF?***

A: KPL SureLINK™ Chromophoric Biotin is soluble in either DMSO or DMF to ~50 mg/mL. As with all NHS esters, exposure to aqueous buffers should be limited to prevent inactivation of the NHS ester by hydrolysis.

***Q: How long can you store rehydrated KPL SureLINK™ Chromophoric Biotin?***

A: It is not recommended that you store KPL SureLINK™ Chromophoric Biotin solution for future use. The 0.5 and 1.0 mg sizes of KPL SureLINK Chromophoric Biotin are designed for single use, although multiple conjugates can be made. If using the 10 mg KPL SureLINK™ Chromophoric Biotin, it is recommended that you weigh the amount of reagent needed instead of dissolving the entire vial.

***Q: Why do I have to store KPL SureLINK™ Chromophoric Biotin under desiccation?***

A: The desiccator helps to remove moisture to prevent inactivation of the NHS ester by hydrolysis.

***Q: My protein normally has an absorbance at 354 nm. Can I still quantitate the amount of biotin?***

A: If your unlabeled protein has an absorbance at 354 nm, you may subtract the portion of the A<sub>354</sub> due to the unlabeled protein if you know the extinction coefficient at 354 nm. Otherwise, use the standard HABA assay to determine biotin incorporation.

***Q: My unlabeled protein contains a protein-based stabilizer such as BSA. How do I prepare my sample for conjugation?***

A: BSA may be removed by commercially available albumin removal kits, such as Bio-Rad Affi-gel blue. If the unlabeled protein and contaminating protein are sufficiently different in size, an appropriate gel filtration column can be used to purify the sample. The contaminating protein can

also be removed by immunoprecipitation, if you have an antibody to recognize the contaminating protein.

**Q: My antibody concentration is less than 0.2 mg/mL. Can I still label my antibody with KPL SureLINK™ Chromophoric Biotin?**

A: Yes, antibodies at a concentration of less than 0.2 mg/mL can still be labeled, but the biotin incorporation rate is low. For best results, concentrate the sample to 0.2 – 5.0 mg/mL using the included spin filters.

**Q: Adding more KPL SureLINK™ Chromophoric Biotin brings the percentage of DMF or DMSO in the conjugation reaction above 5%. Can I still use this molar ratio of biotin?**

A: Yes. Instead of dissolving chromophoric biotin in DMF at 20 mg/mL, dissolve it at 40 mg/mL in order to bring the percentage of DMF in the final reaction to less than 5%.

**Q: Do I have to purify my sample using the spin filters included in the KPL SureLINK™ Chromophoric Biotin Labeling Kit?**

A: No, you do not have to use the included 10,000 MWCO spin filters. The excess biotin can be removed with a variety of desalting columns or dialysis cassettes. For conjugation to antibodies, 50,000 MWCO Vivaspin 6 centrifugal concentrators also work well.

**Q: How much mass will the incorporated biotin add to my protein?**

A: Each mole of KPL SureLINK Chromophoric Biotin will add 695.83 Daltons to the molecular weight of the biotinylated protein.

## TROUBLESHOOTING GUIDE

**Problem 1: During the labeling reaction, the protein precipitates**

Causes and/or Observations	Possible Solution
The percentage of DMF in the conjugation reaction is high.	Dissolve the KPL SureLINK™ Chromophoric Biotin in anhydrous DMF at higher concentration, such as 40 mg/mL. It is preferably to maintain DMF concentrations to less than 5% in the conjugation reaction.

### ***Problem 2: Low biotin incorporation***

<b>Causes and/or Observations</b>	<b>Possible Solutions</b>
The NHS-ester bond of the chromophoric biotin has been hydrolyzed during storage, compromising the labeling reaction.	Upon receipt, store the KPL SureLINK™ chromophoric biotin at 4 °C in a dessicator. Equilibrate biotin vial to room temperature prior to opening to avoid the condensation of moisture.
Contaminants are present in the protein sample that carry a primary amino or a nucleophilic group (ex. Tris, glycine and azide), reducing the efficiency of the modification reaction.	Dialyze the protein sample thoroughly against KPL Modification Buffer prior to the modification step.

### ***Problem 3: Weak Level of Detection in ELISA or Western Blot***

<b>Causes and/or Observations</b>	<b>Possible Solutions</b>
Low signal	Increase the concentration of the protein during the biotinylation reaction. In general, this is more important than the amount of biotin added.
Other proteins are present in the protein sample—compromising the preparation of the desired biotin conjugate.	Fractionate the protein sample over an acrylamide gel electrophoresis and stain using Coomassie dye. Depending on the level of impurity, additional protein purification steps may be required.
Poor recovery of protein after biotinylation using spin filter	If the protein is <10 KDa, it may pass through the spin filter. Use a lower molecular weight cutoff spin filter or dialysis cassette.

### ***Problem 4: High level of Background in ELISA or Western Blot***

<b>Causes and/or Observations</b>	<b>Possible Solutions</b>
The concentration of the biotin conjugate is too high.	Titrate and optimize the amount of conjugate required for each immunoassay.

## RELATED PRODUCTS

Product/Application Group	Product Name	Size	Catalog Number
Protein Labeling Kits & Reagents	KPL SureLINK™ HRP Conjugation Kit	6 x 0.1mg rxn	5610-0022 (84-00-01)
	KPL SureLINK™ HRP Conjugation Kit	6 x 1.0 mg rxn	5610-0023 (84-00-02)
	KPL SureLINK™ HRP Conjugation Kit	2 x 0.1 mg rxn	5610-0024 (84-00-03)
	KPL SureLINK™ AP Conjugation Kit	3 x 0.5 mg rxn	5610-0026 (85-00-02)
	KPL SureLINK™ AP Conjugation Kit	3 x 0.1 mg rxn	5610-0025 (85-00-01)
	KPL Spin-Pure Filters (10K MWCO)	5 per pack	5640-0001 (60-00-53)
Labeled Streptavidin	KPL HRP-labeled Streptavidin	0.5 mg	5270-0029 (14-30-00)
	KPL HRP-labeled Streptavidin, liquid	1.0 mL	5950-0004 (474-3000)
	KPL Phosphatase-labeled Streptavidin	0.5 mg	5270-0030 (15-30-00)
	KPL Phosphatase-labeled Streptavidin, liquid	1.0 mL	5950-0005 (475-3000)
	KPL Fluorescein-labeled Streptavidin	1.0 mg	5270-0028 (072-30-00)
	KPL Cy3-labeled Streptavidin	1.0 mg	5270-0022 (072-01-30-00)
	KPL Cy5-labeled Streptavidin	1.0 mg	5270-0023 (072-02-30-00)
	KPL DyLight 488-labeled Streptavidin	1.0 mg	5270-0024 (072-03-30-00)
	KPL DyLight 680-labeled Streptavidin	1.0 mg	5270-0025 (072-06-30-00)
	KPL DyLight 800-labeled Streptavidin	1.0 mg	5270-0026 (072-07-30-00)
	KPL DyLight 405-labeled Streptavidin	1.0 mg	5270-0027 (072-08-30-00)
	KPL RPE-labeled Streptavidin, liquid	1.0 mL	5270-0021 (0718-30-00)

## REFERENCES

Melnikova, Y. et al., (2000) Antigen-Binding Activity of Monoclonal Antibodies after Incubation with Organic Solvents. *Biochemistry (Moscow)* 65 (11), 1256-1265

Dayhoff, M.O. (1978) *Atlas of Protein Sequence and Structure*, Suppl. 2, National Biomedical Research Foundation, Washington.

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