UV-Tracer™ Maleimide Biotinylation Kit

Description

UV-Tracer™ Maleimide Biotinylation Kit (Cat. # B105-K), provides sufficient reagents for 3 labeling reactions each containing 100-1000 μg of antibody or other thiol-containing protein in a 500 μL reaction volume. This convenient UV traceable biotin labeling reagent permits rapid quantification of incorporated biotin without the need for a HABA biotin assay.

Kit Contents

UV-Tracer™ Biotin Maleimide (Molecular Weight: 1098.30) 3 x 4 mg
Anhydrous DMSO 1 x 10 mL
0.5 M EDTA 1 x 0.1 mL
TCEP-HCL Reducing Agent (M.W. 286.65) 3 x 15 mg
BupH Saline Buffer Pack (BupH™ registered trademark ThermoScientific) 1 packet
Zeba™ Spin Columns (Zeba™ registered trademark ThermoScientific ) 6 x 2 mL

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Introduction

Click Chemistry Tools' UV-Tracer™ Maleimide Biotinylation kit provides all the necessary reagents for the controlled biotinylation of antibodies and other thiol-containing proteins. This advanced labeling reagent contains a UV-traceable benzophenone moiety (350 nm) surrounded by two hydrophilic PEG4 spacer arms (Figure 1). The maleimide functional group targets reduced thioles (-SH) while avoiding modification of susceptible lysine residues. The two hydrophilic PEG4 spacer arms enhance water solubility while reducing the tendency of some proteins to aggregate/precipitate during biotinylation. Extended PEG spacers provide increased conjugate yield while reducing non-specific assay interactions for enhanced assay performance. The traceable chromophore permits rapid determination of incorporated biotin by a
simple measurement at two wavelengths (280 nm/350 nm). UV-Tracer™ Biotin Maleimide reagent is guaranteed to increase labeling reproducibility and yield for maximum assay robustness.

![Chemical structure of UV-Tracer™ Biotin Maleimide](image)

**Figure 1.** Chemical structure of UV-Tracer™ Biotin Maleimide.

### Protein Requirements

- This kit requires 100-1000 µg protein in a fixed volume of 500 µL (e.g. 0.2-2 mg/mL)
- The protein to be biotinylated must be highly purified and its molecular weight known (e.g. 20-200 kDa)
- The protein must have available thiols (e.g. cysteine). If absent sulphhydrils can be added to biomolecules using N-succinimidyl S-acetylthioacetate (e.g. SATA available from ThermoScientific)
- Proteins must be free of reducing agents (e.g. β-ME, DTT or TCEP) prior to labeling with maleimide esters, if present these compounds must be removed (See Material Preparation)
- Buffer exchange columns provided are designed to process 100-1000 µg of protein in a volume 500 ± 50 µL.

### Other Important Considerations

- After reconstitution of UV-Tracer™ Biotin Maleimide reagent in DMSO, use it immediately. Although the maleimide group is more stable than other functional groups, it will hydrolyze to form a nonreactive maleinic acid. Maintain unused stock solution under moisture-free condition (e.g., capped under an inert gas such as argon or nitrogen) at 4°C. Equilibrate reagent vial at room temperature before opening to avoid moisture condensation inside the container.
- Access to a tabletop centrifuge (e.g. Eppendorf 5810 or similar) capable of handling 15 mL conical tubes is required for the spin columns provided.
- In every labeling reaction, the simplified protocol uses a fixed volume (10 µL) of UV Tracer™ Biotin Maleimide/DMSO reagent to label a fixed volume of protein (~ 525 µL). Consequently, the volume of anhydrous DMSO required to dissolve the biotin reagent varies for each labeling reaction (see DMSO volume calculations in Appendix A, Part II)
- The amount of biotin incorporated during the labeling reaction depends primarily on the number and availability of reduced thiols. Generally, a 10- to 20-fold molar excess of reagent is sufficient for most proteins. Over modification of the protein with biotin can affect both function and aqueous stability.
Materials Required but Not Provided

- UV-VIS Spectrophotometer
- Pipettes and tips (P-10 P-100, P-1000)
- 1L beaker
- stir bar with magnetic stir bar
- 6 N HCL
- Table Top Centrifuge (e.g. Eppendorf 5810)
- Ultrapure water (e.g. 18 MΩ-cm)
- 15 mL conical tubes
- Quartz cuvette (500-1000µL)

Material Preparation

A. Preparation of BupH Buffer (pH 6.5)

1. Dissolve the dry-blend BupH buffer pack provided into 500 mL ultrapure water. Adjust the pH of the solution to 6.5 + 0.05 by drop wise addition of 6N HCL. Adjust the final volume to 500 mL with ultrapure water. For long-term storage sterile-filter the solution. Do not add sodium azide or Proclin preservatives as these reagents interfere with protein A280 measurements.

B. Protein Preparation

1. If the lyophilized protein (100-1000 µg) to be biotinylated is pure and free of exogenous thiols (e.g. DTT or β-ME), resuspend in 500 µL BupH buffer (pH 6.5) to obtain a 0.2-2 mg/mL solution, proceed to Protein Reduction (Step E).
2. If the lyophilized protein is known to contain exogenous thiols (e.g. DTT, β-ME) resuspend in 500 µL BupH (pH 6.5) then proceed with buffer exchange (Step C and Step D below), then proceed to Protein Reduction (Step E).
3. If the purified protein to be biotinylated (100-1000 µg) is already in 500 µL of a suitable thiol-free buffer (e.g. MOPS, Tris-HCL, or PBS) at a concentration range from 0.2-2 mg/mL, proceed to Protein Reduction (Step E).

C. Equilibration of Spin Column into BupH (pH 6.5)

1. Twist off the column’s bottom closure and loosen the cap. Place each column into a clean 15 mL conical tube.
2. Centrifuge column at 1,000 x g for 2 minutes to remove storage solution. Place a pen mark on the side of the column where the compacted resin is slanted upward. Place column in centrifuge with the mark facing away from the center of the rotor in all subsequent centrifugation steps. Note-resin will appear white in color and compacted after centrifugation.
3. Add 1 mL BupH buffer (pH 6.5) to the top of each spin column, replace the cap and loosen.
4. Centrifuge at 1000 x g for 2 minutes to remove buffer.
5. Repeat steps 3 and 4 two additional times, discarding buffer from the collection tube after each spin.
6. Transfer the equilibrated spin column (resin appears white and dry) into a clean 15 mL conical tube and immediately proceed with buffer exchange of protein.

D. Buffer Exchange of Protein

1. Buffer exchange protein into BupH (pH 6.5) equilibrated spin column by slowly applying 500 µL protein solution to the center of the equilibrated resin bed.

http://www.clickchemistrytools.com
tel: 480 584 3340
fax: 866 717 2037
2. Centrifuge at 1,000 x g for 2 minutes. Retain the eluate at bottom of 15 mL collection tube. Discard the used spin column.
3. Protein is now buffer exchanged.

E. Reduction of Protein
1. Prior to biotinylation, proteins containing disulfide bonds or free cysteines must be reduced with TCEP to insure proper labeling.
2. Add 500 µL ultrapure water to a vial containing 15 mg TCEP-HCL (105 mM), vortex to dissolve crystals completely.
3. Add 5 µL 500 mM EDTA (provided) to 500 µL protein solution (0.2-2 mg/mL), pipette the mixture up and down several times to mix.
4. Add 25 µL dissolved TCEP (105 mM) to protein solution, pipette up and down several times to mix.
5. Allow the reaction to incubate for 30 minutes.
6. Prepare a buffer exchange spin column as described in Section C.
7. Buffer exchange TCEP reduced protein as described in Section D
8. Immediately after buffer exchange, add 5 µL 500 mM EDTA (provided) to the protein solution , pipette up and down several times to mix.

F. Biotin Labeling Reaction
1. Select how much excess UV-Tracer™ Biotin Maleimide to use during the labeling reaction (e.g. 20-fold excess). Refer to Appendix A, Part I as a reference guide.
2. Determine the volume DMSO required to dissolve UV-Tracer™ Biotin Maleimide reagent using calculations in Appendix A, Part II.
3. Add required volume DMSO to UV-Tracer™ Biotin reagent, vortex vigorously for 2 minutes until reagent is fully dissolved.
4. Add 10 µL biotin/DMSO reagent to protein solution (~ 525 µL) , pipette the mixture up and down several times to mix.
4. Allow labeling reaction to proceed for 1 hour at room temperature.

G. Removal of Excess Biotin Reagent
1. Prepare a buffer exchange spin column as described in Section C.
2. Buffer exchange biotin labeled protein as described in Section D (or buffer of choice).
5. Determine degree of labeling (DOL) and conjugate protein concentration (mg/mL)

H. Degree of Labeling (DOL) and Conjugate Protein Concentration (mg/mL)
1. Measure conjugate's absorbance at 280 nm and 350 nm in a quartz cuvette.
   Note- concentrated protein solutions (e.g. 2 mg/mL) will require dilution of an small aliquot prior to absorbance measurements, while very dilute solutions (e.g. < 0.2 mg/mL) may need to measurement to achieve desired range (e.g. 0.1 to 1.5 AU). A micro-volume spectrophotometer can be used on small aliquots (1-2 µL) without dilution (e.g. Nanodrop®).
2. Calculate the degree of labeling (DOL) and protein concentration with the calculations found in Appendix B, Part I.
## Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor or lower than expected biotinylation of proteins</td>
<td>Incorrect protein concentration and/or possible contaminants in protein sample.</td>
<td>Buffer exchange protein into BupH buffer (pH 6.5) using spin columns provided and confirm concentration of protein prior to labeling.</td>
</tr>
<tr>
<td></td>
<td>Maleimide-ester hydrolyzed</td>
<td>Store UV-Tracer™ Biotin Maleimide reagent at -20°C. Allow product to equilibrate to room temperature before opening.</td>
</tr>
<tr>
<td></td>
<td>Protein has few or no available thiol residues</td>
<td>Avoid buffers that may contain free thiols (β-ME or DTT). Buffer exchange proteins before labeling whenever possible.</td>
</tr>
<tr>
<td></td>
<td>Low A350 absorbance of the biotinylated conjugate</td>
<td>Check spectrophotometer lamp for proper functioning</td>
</tr>
<tr>
<td>Low conjugate yield</td>
<td>Protein may have aggregated/precipitated during biotinylation</td>
<td>Use appropriate relative centrifugal force (e.g. 1000 x g) and recommended spin time to buffer exchange protein. Although rare, some proteins become unstable in aqueous solution on biotinylation and cannot be labeled.</td>
</tr>
</tbody>
</table>
Appendix A

Part I: Excess UV-Tracer™ Biotin Maleimide Reagent to Use in Labeling Reaction

Select the molar excess UV-Tracer™ Biotin Maleimide to use in the labeling reaction. Refer to Table 1 as a reference guide in the selection process. Typical labeling reactions use 5 to 20-fold reagent molar excess depending on initial protein concentration and the number of available thiols. Over modification of antibodies or other proteins with biotin can affect their function and stability.

Table 1

<table>
<thead>
<tr>
<th>Goat IgG (150 kDa)</th>
<th>Molar Equivalents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5x</td>
</tr>
<tr>
<td>(mg/mL)</td>
<td>DOL</td>
</tr>
<tr>
<td>0.2</td>
<td>0.6</td>
</tr>
<tr>
<td>1</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>3.4</td>
</tr>
</tbody>
</table>

Note- Goat IgG possess 32 cysteine residue

Part II: Determine Volume DMSO (µL) Required to Dissolve UV-Tracer™ Biotin Maleimide reagent

1. Calculate millimoles UV-Tracer™ Biotin-Maleimide (M.W. 1098.31) required to label a protein at a desired molar excess \(N_b\), concentration \(C_p\) and volume \(V_p\) with \(MW_p\):

   \[
   \text{mmol reagent required} = N_b \times C_p \times V_p \times \frac{1}{MW_p}
   \]

   \(N_b\) = molar excess UV-Tracer™ Biotin-Maleimide
   \(C_p\) = protein concentration (mg/mL)
   \(V_p\) = volume of protein to be labeled (mL)
   \(MW_p\) = protein molecular weight (Daltons)

2. Calculate microliters anhydrous DMSO required to dissolve UV-Tracer™ Biotin Maleimide reagent:

   \[
   \mu L \ DMSO \ required = \frac{0.0911}{\text{mmol biotin reagent required}}
   \]

See example on next page
**Example 1:** Determine Volume DMSO Required (µL) to Use for the Following Labeling Reaction

To label 0.5 mL of a 2 mg/mL IgG solution (M.W. 150 kDa) with a 20-fold molar excess UV-Trace™ Biotin Maleimide, dissolve reagent (4 mg) into 684.8 µL anhydrous DMSO, then add 25 µL of this stock to antibody solution.

1. Calculate millimoles UV-Trace™ Biotin-Maleimide (M.W. 1098.30) required to label protein at a desired molar excess ($N_b$), concentration ($C_p$) and volume ($V_p$) with $MW_p$:

\[
\text{mmol reagent required} = N_b \times C_p \times V_p \times \frac{1}{MW_p}
\]

\[
\text{mmol reagent required} = 20 \times 2.0 \text{ mg/mL} \times 0.5 \text{ mL} \times \frac{1}{150,000 \text{ dalton}} = 0.000133 \text{ mmol}
\]

\[
\text{mmol reagent required} = 0.000133 \text{ mmol}
\]

2. Calculate volume anhydrous DMSO (µL) required to dissolve UV-Trace™ Biotin NHS (4 mg) as follows:

\[
\mu L \text{ DMSO required} = \frac{\text{mmol reagent per vial} \times 25 \mu L}{\text{mmol reagent required}} = \frac{0.00364 \times 25 \mu L}{0.000133} = 684.8 \mu L
\]

\[
\mu L \text{ DMSO required} = 684.8 \mu L
\]

**Note** - each vial of UV-Trace™ Biotin Maleimide can accommodate 1,600 µL DMSO, if the required volume of DMSO is greater than this nominal volume, transfer the dissolved reagent to a larger vial and add DMSO to achieve the requisite volume.
Appendix B

Part I. Calculate conjugate's DOL (# biotin/protein) and protein concentration (mg/mL) using Equations 1, 2, 3, and 4 below:

\[
\text{Eq. 1} \quad \text{number of biotin per protein} = \frac{\text{molarity biotin}}{\text{molarity protein}}
\]

\[
\text{Eq. 2} \quad \text{molarity of biotin} = \frac{A_{350}}{\epsilon_{350}}
\]

\[
\text{Eq. 3} \quad \text{molarity of protein} = \frac{A_{280c}}{\epsilon_{280}}
\]

\[
\text{Eq. 4} \quad \text{mg/mL} = \frac{A_{280} - (A_{350} \times 0.4475)}{(E1\%/10)} \times \text{dilution factor}
\]

\[A_{350} = \text{conjugate absorbance at 350 ± 5 nm}\]
\[\epsilon_{350} = \text{molar extinction coefficient UV-Tracer™ Biotin} = 19,474 \text{ M}^{-1} \text{cm}^{-1}\]
\[A_{280} = \text{conjugate absorbance at 280 nm}\]
\[A_{280c} = \text{corrected conjugate absorbance at 280 nm} = A_{280} - (A_{350} \times (0.4475))\]
\[\epsilon_{280} = \text{molar extinction coefficient protein (M}^{-1}\text{cm}^{-1}) = \frac{\text{MW}_P \times E1\%}{10}\]

Example 1: Determine DOL and protein concentration for the following biotin labeled protein

A Goat IgG antibody 0.5 mL at 2.0 mg/mL was labeled using a 20-fold molar excess UV-Tracer™ Biotin Maleimide reagent. The conjugate's A280 and A350 (1:4 dilution) was determined to be 0.6739 and 0.2112, respectively. Goat IgG E1% = 13.6 (i.e. 204,000 M\(^{-1}\)cm\(^{-1}\)).

Calculate DOL (# biotin/protein) as follows:

By Equation 2 \quad \text{molarity of biotin} = \frac{0.2112}{19,474 \text{ M}^{-1}\text{cm}^{-1}} = 10.85 \mu\text{M}

By Equation 3 \quad \text{molarity of IgG} = \frac{0.6739 - (0.2112 \times 0.4475)}{204,000 \text{ M}^{-1}\text{cm}^{-1}} = 2.84 \mu\text{M}

By Equation 1 \quad \text{Number of biotin per IgG} = \frac{10.85 \mu\text{M}}{2.84 \mu\text{M}} = 3.82

Calculate conjugate protein concentration (mg/mL)

By Equation 4 \quad \text{mg/mL} = \frac{0.6739 - (0.2112 \times 0.4475)}{1.36} \times 4 = 1.7 \text{ mg/mL}
Appendix C

Figure 2. UV scan (230-450 nm) of Goat IgG (unlabeled control) (A), and UV-Tracer™ Biotin Maleimide labeled Goat IgG (B). Samples were scanned (1:4 dilution) at 0.425 mg/mL in BupH (pH 6.5). The degree of labeling was determined to be 3.8 biotins/IgG.