INSTRUCTIONS

EZ-Link® TFPA-PEG$_3$-Biotin

21303

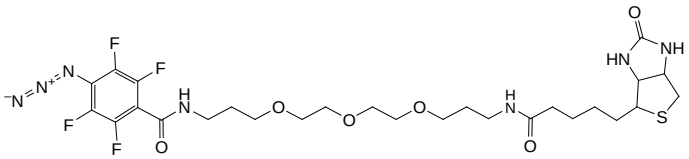
1843.2

Number	Description
21303	**EZ-Link TFPA-PEG$_3$-Biotin**

Package size: 25mg

Molecular Weight: 663.69

Spacer Arm: 33.4Å



Storage: Upon receipt store desiccated at 4°C. Product shipped at ambient temperature.

Introduction

The Thermo Scientific EZ-Link TFPA-PEG$_3$-Biotin enables simple and efficient nonspecific photo-activated labeling of antibodies, proteins and any other molecule that contains C-H or N-H bonds. TFPA-PEG$_3$-Biotin contains the high coupling yield tetrafluorophenyl azide (TFPA) moiety. When exposed to UV light, this perfluorophenyl azide forms an extremely reactive nitrene group that can insert primarily into active carbon-hydrogen bonds (C-H) or add to unsaturated carbon chains. The perfluoroaryl nitrene reacts rapidly and inserts in C-H bonds with greater efficiency than non-fluorinated aryl azides often present in photoactive labeling and crosslinking agents.

The spacer of TFPA-PEG$_3$-Biotin contains a flexible, non-immunogenic hydrophilic polyethylene glycol (PEG) group, which imparts water solubility that is transferred to the labeled molecule. Consequently, antibodies or other proteins labeled with a PEG spacer exhibit less aggregation when stored in solution compared to proteins labeled with reagents having hydrocarbon spacers. By contrast to labeling reagents that contain heterogeneous mixtures of PEG chain lengths, TFPA-PEG$_3$-Biotin is a homogeneous compound of defined molecular weight and spacer arm length, providing greater precision in optimization and characterization of labeling applications.

Biotin is a vitamin that has a high affinity to avidin and streptavidin proteins. Biotinylated proteins typically retain biological activity because the biotin group is relatively small. An antibody conjugated with several biotin molecules can amplify signal thereby increasing the sensitivity of many assays. The biotin-avidin interaction is strong and, once formed, is unaffected by most extremes of pH, organic solvents and other denaturing agents. Labeled proteins can be purified using immobilized avidin, streptavidin, or Thermo Scientific NeutrAvidin Protein affinity resins and detected in ELISA, dot blot or Western blot applications.

Important Product information

Reconstitution and Stability

- TFPA-PEG$_3$-Biotin is light-sensitive. Equilibrate vial to room temperature protected from light before opening.
- TFPA-PEG$_3$-Biotin is most soluble in DMF or DMSO. For best results, solubilize the reagent in one of these solvents before adding it to an aqueous protein solution.
- Discard any unused reconstituted biotin-labeling reagent.

Photoactivation Information

- Use a shallow reaction vessel for maximum efficiency. Irradiation efficiency decreases as the distance between the reactants and the light source increases. Choose a low protein-binding vessel for maximum sample recovery.
- Use a UV lamp that irradiates at 300-370nm (see Note below) for photoactivation. High-wattage lamps are more effective and require shorter exposure times than low-wattage lamps. Suggestions for lamps include the Stratalinker 2400 (5 × 15 watt bulbs, emission at either 312nm or 365nm), mercury vapor lamps (180 watt, 350 watt, between 300nm and 360nm), XeCl excimer laser (150mJ, 308nm) and UV Spectroline lamps (medium/long wavelength lamps). Using low-wattage hand-held lamps, such as 6 watt lamps, will result in lower conjugation efficiencies.

Note: The optimal wavelength for photoactivation is 320nm. Avoid UV lamps that emit light at 254nm because this wavelength causes proteins to photodestruct. Filters that remove light at wavelengths below 300nm are ideal. Using a second filter that removes wavelengths above 370nm can be beneficial, but is not essential.

- Position the UV lamp 5-10cm from the reaction. For lamps > 150 watts, use a distance of 10cm. For lower-powered lamps, use a distance of 5cm. Perform photoactivation by placing the lamp above the open reaction vessel to avoid impeding irradiation by the vessel.

Example Procedure for Protein Biotinylation

Note: Conduct all reagent manipulations in subdued light or in the dark to protect the perfluoroaryl-azide moiety from early photoactivation. Wrap reaction tube in foil or protect from direct light by some other means.

A. Materials Required

- Phosphate-buffered Saline (PBS) containing 0.1M phosphate, 0.15M sodium chloride; pH 7.2 (Thermo Scientific BupH Phosphate Buffered Saline Packs, Product No. 28372) or other amine-free buffer at pH 7.0-8.0

  Note: For photo-activation any buffer at pH 7-9 may be used provided it is devoid of compounds that contain C-H or N-H bonds.

- Dialysis unit such as Thermo Scientific Slide-A-Lyzer MINI Dialysis Unit or a desalting column such as the Thermo Scientific Zeba Spin Desalting Columns (i.e., Product No. 89889 for 200-700µL samples or Product No. 89891 for 500-2000µL samples) or other product for buffer exchange

B. Calculations

The amount of biotin reagent to use for each reaction depends on the amount of the protein to be labeled and the protein concentration. By using the appropriate molar ratio of biotin to the protein, the extent of labeling can be controlled. For dilute protein solutions (e.g., 2mg/mL) a greater fold molar excess of biotin is used compared to a concentrated protein solution (e.g., 10mg/mL). For example, for best results use ≥ 20-fold molar excess of biotin for a 2mg/mL IgG solution or ≥ 12-fold molar excess of biotin for a 10mg/mL IgG solution.

1. Calculate millimoles of TFPA-PEG_3-Biotin to add to the reaction for a 20-fold molar excess:

$$\text{ml protein} \times \frac{\text{mg protein}}{\text{ml protein}} \times \frac{\text{mmol protein}}{\text{mg protein}} \times \frac{\text{20 mmol Biotin}}{\text{mmol protein}} = \text{mmol Biotin}$$

   - 20 = Recommended molar fold excess of biotin reagent per protein sample

2. Calculate microliters of 10 mg/ml TFPA-PEG_3-Biotin (prepared in Step C.3) to add to the reaction:

$$\text{mmol Biotin} \times \frac{\text{664 mg}}{\text{mmol Biotin}} \times \frac{\text{200 µl}}{\text{2 mg}} = \text{µl Biotin Solution}$$

   - 664 = Molecular weight of TFPA-PEG_3-Biotin
   - 200 = Microliters of solvent in which 2 mg of TFPA-PEG_3-Biotin is dissolved

Example: For 1ml of a 2mg/mL IgG (150,000 MW) solution, ~17.7µL of 10mg/mL TFPA-PEG_3-Biotin will be added.

$$\text{1 ml IgG} \times \frac{\text{2 mg IgG}}{\text{1 ml IgG}} \times \frac{\text{1 mmol IgG}}{\text{150,000 mg IgG}} \times \frac{\text{20 mmol Biotin}}{\text{1 mmol IgG}} = \text{0.000266 mmol Biotin}$$

$$\text{0.000266 mmol Biotin} \times \frac{\text{664 mg}}{\text{mmol Biotin}} \times \frac{\text{200 µl}}{\text{2 mg}} = \text{17.7 µl Biotin Solution}$$

C. Biotinylation Reaction

1. Dissolve or dilute the protein from 0.1 to 10mg/mL in PBS. Typical reaction volumes are from 100µL to 500µL.
2. Transfer the protein to an amber or foil-covered microcentrifuge tube.
3. Calculate amount of reagent needed to achieve 2- to 50-fold molar excess over protein. (see Section B)
4. Prepare a 10mg/mL solution of TFPA-PEG_3-Biotin in DMF or DMSO. To the protein, add the volume of reagent necessary for the desired molar excess, mix gently and let stand in the dark for 2 minutes before activating.

   Note: To minimize detrimental affects to the protein, do not exceed 15% of organic solvent in the reaction.
5. Photoactivate the perfluoroaryl azide moiety with a UV light source (see the Photoactivation Information Section). For best results, place samples on ice when using high-wattage lamps to prevent sample heating. The optimal photo-activation wavelength is 320nm. Effective exposure time varies depending on the intensity of the light source. Suggested exposure times are as follows:

   • 8 minutes for a device with 2 × 15 watt lamps at a distance of 5cm
   • 5 minutes for a device with 5 × 15 watt lamps at a distance of 5cm
   • 5 minutes for a 180 watt lamp at a distance of 10cm
   • 1.5 minutes for a 350 watt lamp at a distance of 10cm
6. Remove non-reacted reagent by dialyzing against PBS for at least 4 hours at room temperature or overnight at 4°C. Place the dialysis container in the dark. Alternatively, use a Zeba™ Spin Desalting Column to remove non-reacted reagent.
7. For storage, divide the labeled protein into single-use aliquots and store protected from light at -80°C. Avoid multiple freeze/thaw cycles of the protein.

Information Available from the Web

Please visit our website for additional information including the following items:

- Tech Tip #11: Light sources and conditions for photoactivation of aryl azide crosslinking reagents
- Tech Tip #43: Protein stability and storage
- Tech Tip #6: Extinction coefficient guide

Related Thermo Scientific Products

89882	**Zeba Spin Desalting Columns, 0.5mL,** 25 columns, for 30-130µL samples
89889	**Zeba Spin Desalting Columns, 2mL,** 5 columns, for 200-700µL samples
89891	**Zeba Spin Desalting Columns, 5mL,** 5 columns, for 500-2000µL samples
89893	**Zeba Spin Desalting Columns, 10mL,** 5 columns, for 700-4000µL samples
69550	**Slide-A-Lyzer® MINI Dialysis Unit,** 3.5K MWCO, 50 units
69560	**Slide-A-Lyzer MINI Dialysis Unit**, 7K MWCO, 50 units
69570	**Slide-A-Lyzer MINI Dialysis Unit,** 10K MWCO, 50 units
28372	**BupH™ Phosphate Buffered Saline Packs,** 40 each
20673	**Dimethylformamide (DMF),** 50mL
20688	**DMSO (Dimethylsulfoxide),** 950mL

References

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Pandurangi, R.S., *et al.* (1997). Chemistry of bifunctional photoprobes: Part 1. Perfluoro azido functionalized phosphorus hydrazides as novel photoreactive heterobifunctional chelating agents: High efficiency nitrene insertion on model solvents and proteins. *J. Org Chem*. **62(9):**2798-807.

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Yan, M., *et al.* (1994). *N*-Hydroxysuccinimide ester functionalized perfluorophenyl azides as novel photoactive heterobifunctional cross-linking reagents. The covalent immobilization of biomolecules to polymer surfaces. *Bioconjugate Chem.* **5:**151-7.

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Current versions of product instructions are available at *www.thermoscientific.com/pierce*. For a faxed copy, call 800-874-3723 or contact your local distributor.