INSTRUCTIONS

EZ-Link® Psoralen-PEG$_3$-Biotin

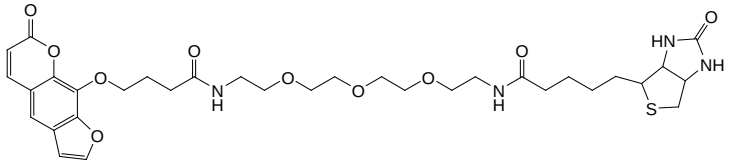
29986

0744.4

Number	Description
29986	**EZ-Link Psoralen-PEG$_3$-Biotin,** 5mg

Molecular Weight: 688.79

Spacer Arm: 36.86Å



Storage: This product is shipped at ambient temperature. Upon receipt store product at 4°C protected from light and moisture.

Introduction

Thermo Scientific EZ-Link Psoralen-PEG$_3$-Biotin contains a water-soluble polyethylene glycol (PEG) spacer arm that produces better solubility of biotinylated molecules than aliphatic long-chain spacers. The extended spacer provides maximum accessibility for detection of molecules labeled with biotin binding proteins. Unlike other aliphatic, long-chain biotinylation reagents, the PEG spacer will not become buried or hidden in hydrophobic pockets. Also, modifications performed with this unique biotin derivative will not decrease the solubility of biotinylated molecules, but actually promotes conjugate solubility.

The photoreactive psoralen group provides much greater covalent insertion yields than phenyl azide-containing compounds, making this regent a superior alternative to typical photoactivatable biotin derivatives. The psoralen tricyclic planar ring system intercalates into double-stranded DNA, RNA, PCR products, cDNA, oligonucleotides and to a lesser extent single-stranded DNA. Psoralen-PEG$_3$-Biotin can be used with gel-purified oligonucleotides because agarose does not interfere with conjugation. High-yield conjugation occurs after a brief exposure to UV light (>350nm, 10-30 minutes. The nucleic acid labeling site of the psoralen group (the 5,6 double bond in thymine residues) does not interfere with subsequent hybridization reactions, creating a highly sensitive direct labeling alternative to radioactive probes.

Example Procedure for Biotinylating DNA

The following protocol is an adaptation from Henriksen, *et al*. For best results, empirically optimize each specific application and experimental system.

1. Adjust DNA or RNA to the desired concentration (e.g., 20-100µg/mL) in TE buffer (10mM Tris, 1mM EDTA, pH 7.4).

   Note: The psoralen group can crosslink the two strands of double-stranded DNA. Before labeling, heat-denature double-stranded DNA to produce single-stranded DNA. To denature, boil DNA for 5 minutes and then place tube in a dry ice/ethanol bath to quickly cool the denatured DNA.

2. In subdued indirect light, dissolve Psoralen-PEG$_3$-Biotin in DMF or DMSO to 20mM (~1.38mg/100µL).

3. Add the biotin solution to the DNA or RNA and mix well. In a standard reaction, the final concentration of Psoralen-PEG$_3$-Biotin is ~200µM (1:100 dilution of stock solution prepared in Step 2). A serial dilution of the Psoralen-PEG$_3$-Biotin stock solution may be required when using small reaction volumes.

4. Irradiate the open reaction tube from above using a long wavelength UV light source for at least 10-30 minutes. If desired, keep the sample cooled on ice during irradiation.

5. To remove non-reacted biotin, precipitate sample with 0.2M potassium acetate and two volumes of ethanol. After centrifugation, wash pellet with 70% ethanol and allow it to dry. Dissolve the biotinylated sample in water or buffer.

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Determination of Biotin Incorporation

Biotin incorporation can be estimated using the HABA (4´-hydroxyazobenzene-2-carboxylic acid) method. In solution, the HABA dye binds avidin, forming a complex with maximal absorption at 500 nm. When biotin is added to the solution, its higher affinity for avidin displaces the HABA and the absorption at 500 nm decreases proportionately. The absorbance of the HABA-avidin solution is measured before and after adding the biotin-containing sample. The change in absorbance relates to the amount of biotin in the sample. The Thermo Scientific Pierce Biotin Quantitation Kit (Product No. 28005) contains a biotinylated protein control and a premix of HABA and avidin supplied in convenient No-Weigh™ Microtube packaging.

Related Thermo Scientific Products

95034, 95035	**3UV Lamps (254nm, 302nm, 365nm settings)**
69576	**Slide-A-Lyzer® MINI Dialysis Unit Kit, 10K MWCO, 0.1mL,** 10/pkg
66382, 66807	**Slide-A-Lyzer Dialysis Cassette Kits, 10K MWCO,** for 0.5-3mL and 3-12mL sample volumes, respectively
28005	**Pierce® Biotin Quantitation Kit**
20347	**Streptavidin Agarose Resin,** 2mL
20228	**Pierce Monomeric Avidin Kit**
21115	**Pierce Biotinylated Protein Interaction Pull-Down Kit**
21126	**Streptavidin, Horseradish Peroxidase Conjugated,** 1mg
21324	**Streptavidin, Alkaline Phosphatase Conjugated,** 1mg
15120	**Streptavidin Coated Strip Plates,** 5 plates
21330	**EZ-Link NHS-PEG$_4$-Biotin**, 25mg
21331	**EZ-Link Sulfo-NHS-SS-Biotin,** 100mg
21325	**EZ-Link NHS-Chromogenic Biotin,** 10mg

General References

Henriksen, U., *et al*. (1991). Azidobenzoyl-, azidoacridinyl-diazocyclopentadienyl-carbonyl-, and 8-propyloxypsoralen photobiotinylation reagents. Syntheses and photoreactions with DNA and protein. *Photochem Photobiol A Chem* **57:**331-42.

Oser, A., *et al*. (1988). Sensitive non-radioactive dot-blot hybridization using DNA probes labeled with chelate group substituted psoralen and quantitative detection by europium ion fluorescence. *Nucl Acid Res* **16:**1181-96.

Wassarman, D.A. (1993). Psoralen crosslinking of small RNAs *in vitro*. *Molec Biol Reports* **17:**143-51.

Product Reference

Zhang, Y., *et al*. (2001). Reproducible and inexpensive probe preparation for oligonucleotides arrays. *Nucl Acid Res* **29(13):**E66-6.

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