User Manual

Uni-Link™ AminoModifier User Manual

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I. Introduction

Description

Uni-Link™ AminoModifier (Figure 1) is a cyanoethyl phosphoramidite that directly incorporates primary aliphatic amines into oligonucleotides (Figure 2).

Features

- Uni-Link AminoModifier is fully compatible with any commercial DNA synthesizer.
- Uni-Link AminoModifier has a 7 atom spacer arm.
- The unique structure of Uni-Link Aminomodifier allows for primary amine incorporation at the 5' terminus or any internal position of the oligonucleotide while maintaining the natural internucleotide phosphate distance (Figure 2).
- By repetitive coupling cycles, multiple primary amine modification can be achieved.
- Uni-Link AminoModifer possess a dimethoxytrityl (DMT) hydroxyl protection group that allows for easy coupling efficiency quantitation.
- The base-labile fluorenylmethyl carbamate (Fmoc) amine protecting group is removed during ammonium hydroxide treatment; therefore, no extra deprotecting steps are necessary.
- The aminomodified oligonucleotide can be labeled with any amine-reactive molecule, for example, biotin-NHS ester or FITC.

I. Introduction

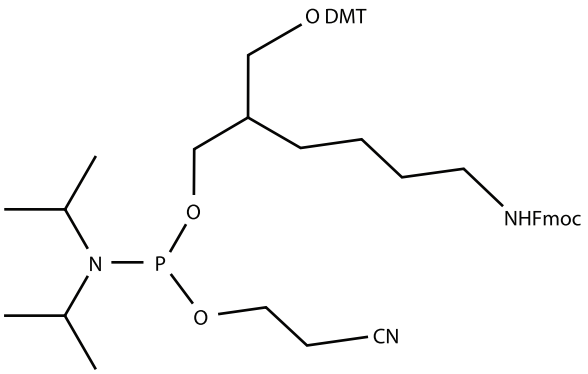

Figure 1. Structural diagram of Uni-Link AminoModifier, MW 873.

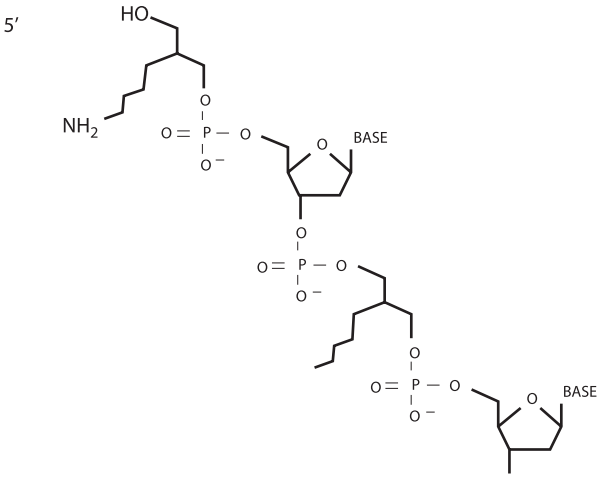

Figure 2. Multiple aminomodification of an oligonucleotide with Uni-Link AminoModifier. Uni-Link AminoModifier is shown incorporated at the 5' terminus and an internal site.

II. Protocols

Note: Uni-Link AminoModifier should be stored at –20°C, desiccated.

A. Protocol for Automated DNA Synthesizers

1. Dissolve 100 mg Uni-Link AminoModifier in 1.1 ml *anhydrous* acetonitrile to give a concentration of 0.1 M. Dissolve 250 mg in 2.8 ml *anhydrous* acetonitrile.

   Note: Make sure the Uni-Link AminoModifier is completely dissolved before proceeding to Step 2.
2. Transfer the solution to the extra phosphoramidite port on your DNA synthesizer. (Uni-Link AminoModifier is supplied in an ABI industrial standard vial.) It is recommended to make all transfers of anhydrous acetonitrile with a syringe for ease of handling and for minimum exposure to air. Uni-Link AminoModifier is stable for two days after dissolving in acetonitrile.
3. Enter in the oligonucleotide sequence you wish to synthesize. You may program Uni-Link AminoModifier to couple at any nucleotide position in your oligonucleotide. Multiple Uni-Link AminoModifer units can be added by programming multiple coupling cycles. It is best to separate adjacent Uni-Link AminoModifer sites by at least one normal nucleotide. This is beneficial for subsequent labeling and detection.
4. Carefully prime the Uni-Link AminoModifier line on the DNA synthesizer. The line must be well-primed to obtain optimum coupling efficiency.
5. Initiate the synthesis using the **TRITYL-OFF** mode, i.e., remove the final DMT group.
6. If desired, the coupling efficiency of Uni-Link AminoModifier can be determined by measuring the dimethoxytrityl cation concentration according to your manufacturer's recommended protocol. Coupling efficiencies can range depending on the performance of your instrument.

B. Deprotection and Cleavage

1. Cleave the aminomodifed oligonucleotide from the solid support by treating with ammonium hydroxide at room temperature for 1.5 hr. It is convenient to use luer tip syringes for this step.
2. Complete the deprotection by transferring the ammonium hydroxide to a 1.5 ml screw cap microcentrifuge tube. Heat at 55°C for 6 hrs. **Caution:** Ammonia gas builds up pressure at 55°C in a closed reaction vessel; cool to 4°C before opening screw cap microcentrifuge tube.
3. Evaporate to dryness by vacuum centrifugation or rotary evaporation.
4. Store at –20°C.

II. Protocols *continued*

C. Measurement of Primary Aliphatic Amines

The measurement of the incorporation of each aliphatic primary amine unit in the oligonucleotide is best done by measuring the dimethoxytrityl cation concentration from your synthesizer according to step A.6. The percentage coupling efficiency of Uni-Link AminoModifer is equal to the percentage of the incorporated amine.

D. Labeling Aminomodified Oligonucleotides

Any amine-reactive reagent may be used to label an aminomodified oligonucleotide. Protocols for attachment of Biotin-XX-NHS Ester (Cat. No. 635805) and fluorescein isothiocyanate (FITC) are given below.

Biotin Labeling

1. Prepare an 80 mg/ml solution of Biotin-XX-NHS Ester in N,N-dimethylformamide (DMF) by following the exact procedure below. This solution is enough for two biotin labeling reactions of 100 µl per reaction. This procedure can be scaled up proportionally for more than two reactions.
   a. Preheat heating block to 95°C.
   b. Thaw Biotin-XX-NHS Ester to room temperature.
   c. Transfer 250 µl of DMF to a 1.5 ml screw cap microcentrifuge tube.
   d. Using an analytical balance, carefully weigh out 20 mg of Biotin-XX-NHS Ester onto a small piece of weighing paper.
   e. Transfer Biotin-XX-NHS Ester to the 1.5 ml microcentrifuge tube, screw cap on tightly, and quickly vortex to get a suspension in DMF.
   f. Place in heating block at 95°C for *exactly* 3 min. Vortex until the Biotin-XX-NHS Ester has completely dissolved.
2. Reconstitute the aminomodified oligonucleotide (1.0 µmol) in 400 µl of 0.5 M sodium bicarbonate, pH 8.5. Vortex to get a complete solution.
3. Add 100 µl of the 80 mg/ml Biotin-XX-NHS Ester solution to the aminomodified oligonucleotide solution. **Quickly vortex to achieve a complete solution.**
4. React at 40°C for 4 hrs.

FITC Labeling

1. Prepare a 100 mg/ml solution of FITC in DMF by following the exact procedure below. This solution is enough for two FITC labeling reactions of 100 µl per reaction This procedure can be scaled up proportionally for more than two reactions.

II. Protocols *continued*

a. Transfer 250 µl of DMF to a 1.5 ml screw cap microcentrifuge tube.

b. Using an analytical balance, carefully weigh out 25 mg of FITC onto a small piece of weighing paper.

c. Transfer FITC to the 1.5 ml microcentrifuge tube, screw cap on tightly, and quickly mix by vortexing until all the FITC is in solution.

2. Reconstitute the aminomodified oligonucleotide (1.0 µmol) in 400 µl of 0.5 M sodium carbonate/bicarbonate (Na_2CO_3/$NaHCO_3$), pH 9. Vortex to get a complete solution.

3. Add 100 µl of the 100 mg/ml FITC ester solution to the aminomodified oligonucleotide solution. **Quickly vortex to achieve a complete solution.** There may be a slight precipitate.

III. Tips for Achieving High Coupling Efficiency

Uni-Link AminoModifier has been rigorously quality control tested to ensure high performance. However, there are certain details that must be considered when using Uni-Link AminoModifier. We recommend reading the following tips before using this reagent.

1. Be sure your DNA synthesizer is coupling at ≥ 97% for normal phosphoramidites. This is easily accomplished by measuring the trityl concentration.
2. Be sure to use *anhydrous* acetonitirile when dissolving Uni-Link AminoModifier.
3. Be sure to prime the Uni-Link AminoModifier line well before initiating synthesis.
4. Use Uni-Link AminoModifier within two days after dissolving in acetonitrile and loading onto synthesizer. The best results are achieved when Uni-Link AminoModifier is used immediately after dissolving in anhydrous acetonitrile.
5. If possible, extend coupling times to 5–10 min to ensure high coupling efficiency.

IV. References

1. Nelson, P., *et al.* (1989) *Nucleic Acids Res.* **17**:7179.

V. Related Products

For a complete listing of all Clontech products,
please visit **www.clontech.com**

Product	Cat. No.
• N-MMT-C_6 AminoModifier	635819
• N-MMT-C_{12} AminoModifier	635821
• AminoModifier II	635820
• N-TFA-C_6-AminoModifier	635822
• 3' Amine-ON CPG	635828
• DMT-C_6-3' Amine-ON CPG	635829
• Biotin-XX-NHS Ester	635805

Notes