



GLEN RESEARCH

22825 DAVIS DRIVE
STERLING, VIRGINIA
20164

PHONE

703-437-6191

800-327-GLEN

FAX

703-435-9774

INTERNET

WWW.GLENRES.COM

CHEMICAL PHOSPHORYLATION REAGENT

INTRODUCTION

The use of this reagent is an alternative to enzymatic techniques for oligonucleotide phosphorylation¹ with the advantage of allowing determination of phosphorylation efficiency.

USE OF CHEMICAL PHOSPHORYLATION REAGENT

Diluent: Anhydrous Acetonitrile

Add fresh diluent to product vial to recommended concentration and swirl vial occasionally over several minutes until product is completely dissolved. (Some oils may require between 5 and 10 minutes.) Use care to maintain anhydrous conditions. In case of transfer to an alternate vial type, ensure recipient vial has been pre-dried. For more information, see: http://www.glenresearch.com/Technical/TB_ABITransfer.pdf.

Coupling: No changes needed from standard method recommended by synthesizer manufacturer.

Deprotection: Deprotect as required by nucleobases.

Storage: Refrigerated storage, maximum of 2-8 °C, dry

Stability in Solution: 2-3 days

USE OF 3'-PHOSPHATE CPG OR PS

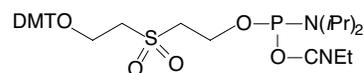
Coupling: This support should be used in a manner identical to normal protected nucleoside support since it contains the DMT group.

Deprotection: Cleavage of the oligonucleotide from this support requires 2 hours at room temperature with ammonium hydroxide. Complete the deprotection using the protocol required by the nucleobases.

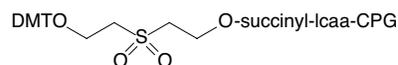
Note: complete deprotection to the 3'-phosphate requires a minimum of 17 hr @ RT or 4 hr @ 55 °C in ammonium hydroxide, 30 minutes @ 65 °C in AMA, or 8 hr @ RT in K₂CO₃/MeOH

Storage: Freezer storage, -10 to -30°C, dry

FIGURE 1: STRUCTURES OF CHEMICAL PHOSPHORYLATION REAGENT



10-1900: Chemical Phosphorylation Reagent



20-2900: 3'-Phosphate CPG
26-2900: 3'-Phosphate PS

KEY POINTS

- The DMT-group is removed during deprotection and thus is not available for DMT-on purification.
- These reagents can only be used to make 3' or 5' monophosphates. They are not useful for the synthesis of polyphosphates.
- The 3'-Phosphate supports are not compatible with the β-elimination technique using 10% diethylamine in acetonitrile to remove cyanoethyl groups without significant yield loss. Use 3'-CPR II CPG (20-2903) instead.
- When using AMA for deprotection, complete elimination to the 3'- or 5'-phosphate requires 30 minutes @ 65 °C.

5'-PHOSPHORYLATION

The DMT group should be removed on the synthesizer by the standard deblocking method to determine coupling efficiency, if desired. Standard deprotection is used to cleave the modified oligonucleotide from the support and to remove all other protecting groups as well as the sulfonylethyl group. Note that the DMT group is eliminated with the sulfonylethyl group during ammonium hydroxide deprotection, rendering this product incompatible with reverse phase chromatographic purification techniques.

3'-PHOSPHORYLATION

Using Chemical Phosphorylation Reagent

Chemical Phosphorylation Reagent has proved to be fast and convenient for chemical phosphorylation of the 5'-terminus of oligonucleotides. In addition, this reagent has proved its utility for simple phosphorylation of the 3'-terminus. It is introduced as the first addition to any nucleoside support, followed by normal synthesis of the target oligonucleotide. After the standard deprotection, the linkage decomposes and is β -eliminated from the target molecule, leaving a phosphate group at the 3'-terminus.

Using 3'-Phosphate CPG or PS

A simple approach to 3'-phosphorylation is to use 3'-phosphate CPG or PS. In this case, the 3'-nucleotide derives from the first nucleoside phosphoramidite addition.

PURIFICATION

5'-Phosphates

Oligonucleotides with 5'-phosphates may be purified using either HPLC or electrophoresis. Ion-exchange HPLC or polyacrylamide gel electrophoresis, using conventional methods, are recommended. If chromatographic purification is considered to be unnecessary, the phosphorylated oligonucleotide can be conveniently desalted on a purification cartridge. However, the simplest method is to use Chemical Phosphorylation II (10-1901) or Solid CPR II (10-1902) and purify using an RP purification cartridge (e.g., Glen-Pak™) or RP HPLC.

3'-Phosphates

For 3'-phosphorylated oligonucleotides, the final DMT group may be removed on the synthesizer or it may be retained to aid in purification. If the DMT group is retained, it may be removed on a purification cartridge or, following purification, by treating the oligonucleotide with acetic acid:water (20:80) at room temperature for 1 hour.

REFERENCE

1. T. Horn and M. Urdea, *Tetrahedron Lett.*, 1986, **27**, 4705.