CHEMICAL PHOSPHORYLATION REAGENT

INTRODUCTION

The use of this reagent is an alternative to enzymatic techniques for oligonucleotide phosphorylation 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

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**