

## Chemical phosphorylation reagent

<http://www.lumiprobe.com/p/chemical-phosphorylation-reagent-ii>

Chemical phosphorylation reagent for the synthesis of 5'-phosphorylated oligonucleotides. The reagent contains a DMT group in its structure, which allows oligonucleotide purification on C18-cartridges or by reversed-phase chromatography. To yield 5'-phosphorylated oligonucleotide, remove DMT group and then deprotect the phosphate group with diluted ammonium solution (0.1 M). Deprotection of the phosphate group is rapid and efficient even under mild basic conditions, so this reagent can be used for DMT-Off synthesis, for example for RNA synthesis, or with dye phosphoramidites that require mild deprotection conditions.

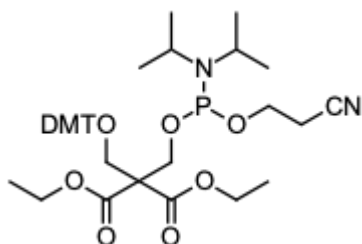
## Usage

Diluent: anhydrous acetonitrile. Add the diluent to the recommended concentration (0.1 M) and wait until reagent dissolution is complete while mixing periodically. This reagent is viscous amorphous, and it may take up to 10 minutes to dissolve it. Store the diluted reagent in anhydrous conditions for not more than 24 hours.

**Coupling:** 6 minutes

## Deprotection:

1. DMT group is removed during synthesis: to yield the 5'-terminal phosphate, deprotect under standard conditions using ammonium hydroxide.
2. DMT-ON, cartridge purification: standard cartridge purification conditions. After elution from the cartridge, deprotect the phosphate group by adding an equivalent volume of 25% aqueous ammonium solution to oligonucleotide solution, incubate for 15 minutes at room temperature, and dry the oligonucleotide down.
3. DMT-ON, purification by HPLC: purify 5'-DMT-oligonucleotide by reversed-phase HPLC. To remove DMT group, redissolve oligonucleotide in 80% acetic acid and incubate for 30 minutes at room temperature. Dry the oligonucleotide down, add 10% aqueous ammonium and incubate for 15 minutes to cleave the remaining fragment of the protecting group.
4. DMT-OFF: to yield the 5'-phosphate, use standard deprotection conditions after synthesis.



**Structure of chemical phosphorylation reagent**

### General properties

Appearance:	colorless semisolid
Molecular weight:	722.8
CAS number:	171285-25-9
Molecular formula:	C <sub>39</sub> H <sub>51</sub> N <sub>2</sub> O <sub>9</sub> P
IUPAC name:	Propanedioic acid, 2-[[bis(4-methoxyphenyl)phenylmethoxy]methyl]-2-[[[bis(1-methylethyl)amino](2-cyanoethoxy)phosphino]oxy]methyl]-, 1,3-diethyl ester
Quality control:	NMR <sup>1</sup> H, <sup>31</sup> P, HPLC-MS (95%)

Storage conditions: Storage: 12 months after receipt at -20°C in the dark. Transportation: at room temperature for up to 3 weeks. Avoid prolonged exposure to light. Desiccate.

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### **Oligo synthesis details**

Diluent: acetonitrile

Coupling conditions: recommended coupling time 6 min

Deprotection conditions: identical to protected nucleobases