

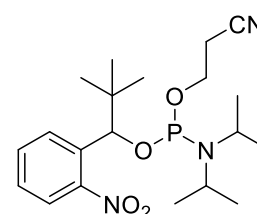
Achieving High-Purity Oligonucleotides Synthesis

5'-Monophosphorylation Reagent

This reagent is an innovative chemical phosphorylation reagent that enables the synthesis of high-purity 5'-monophosphorylated oligonucleotides using the phosphoramidite method, a standard approach in chemical oligonucleotide synthesis. By introducing a hydrophobic nitrobenzyl (Nb) tag, enables efficient separation and purification of the target oligonucleotide by reverse phase HPLC using the same principle as DMT-ON purification, resulting in markedly improved purity. Moreover, the ultraviolet (UV)-induced deprotection step suppresses degradation of unstable RNA, making this reagent suitable for synthesizing 5'-monophosphorylated RNA.

5'-monophosphorylated RNA obtained using this technology can be applied to chemically synthesized mRNA and circular mRNA, and is expected to contribute significantly to the advancement of RNA-based therapeutics research.

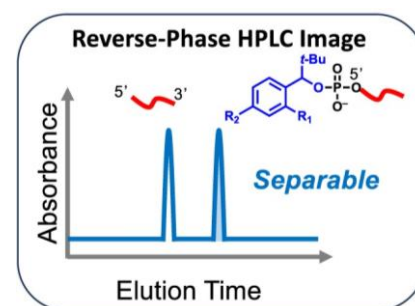
FUJIFILM Wako has added to its product lineup a new 5'-monophosphorylation reagent developed by Professor Abe's research group at Nagoya University.



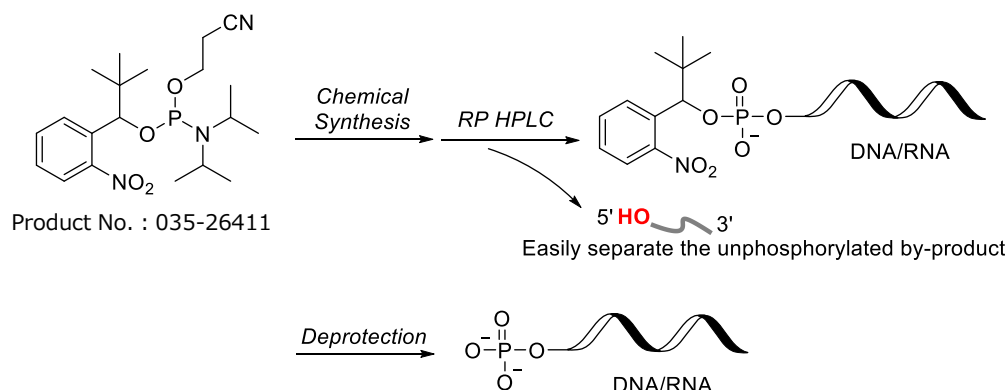
5'-monophosphorylation reagent

Features

- Chemical phosphorylation reagent with a nitrobenzyl tag
- Excellent coupling efficiency, up to 99%
- Enables high-purity isolation of oligos
- Innovative photolabile hydrophobic tag removed by UV irradiation



Application

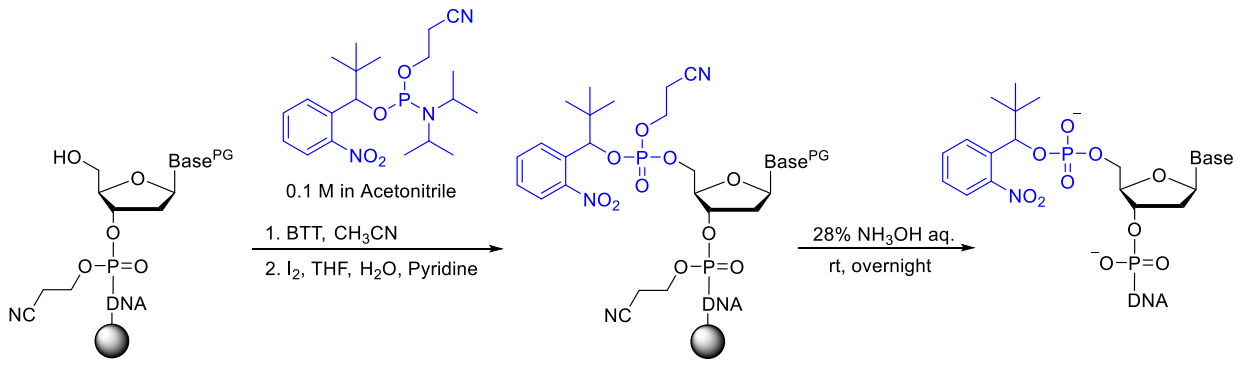


Product No.	Product Name	Grade	Pkg. Size	Storage Condition
		CAS RN®		
035-26411	2-Cyanoethyl [2,2-dimethyl-1-(2-nitrophenyl)propyl] N,N-Diisopropylphosphoramidite	for Nucleic Acid Synthesis	250 mg	Keep at -20 degrees C.
		2892148-78-4		

This product is covered by Patent based on WO2023/282245 and licensed from Crafton Biotechnology.

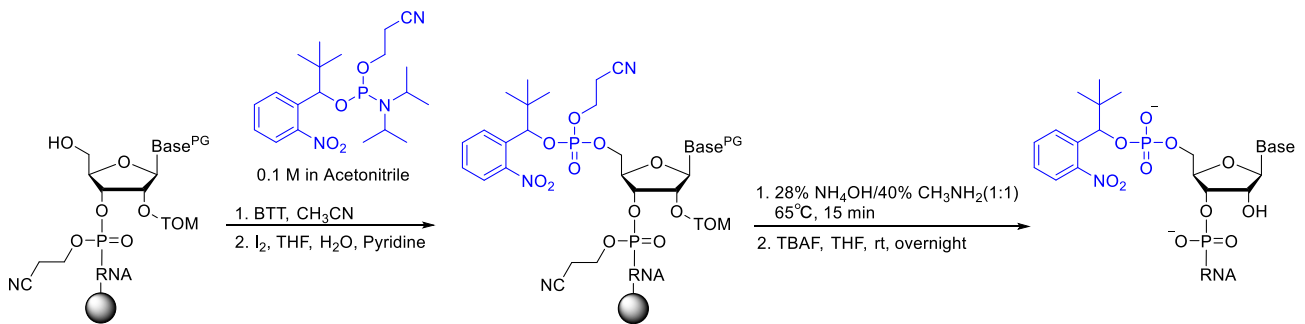
Synthesis of 5'-monophosphorylated oligonucleotides

(i) Synthesis of 5'-monophosphorylated DNAs



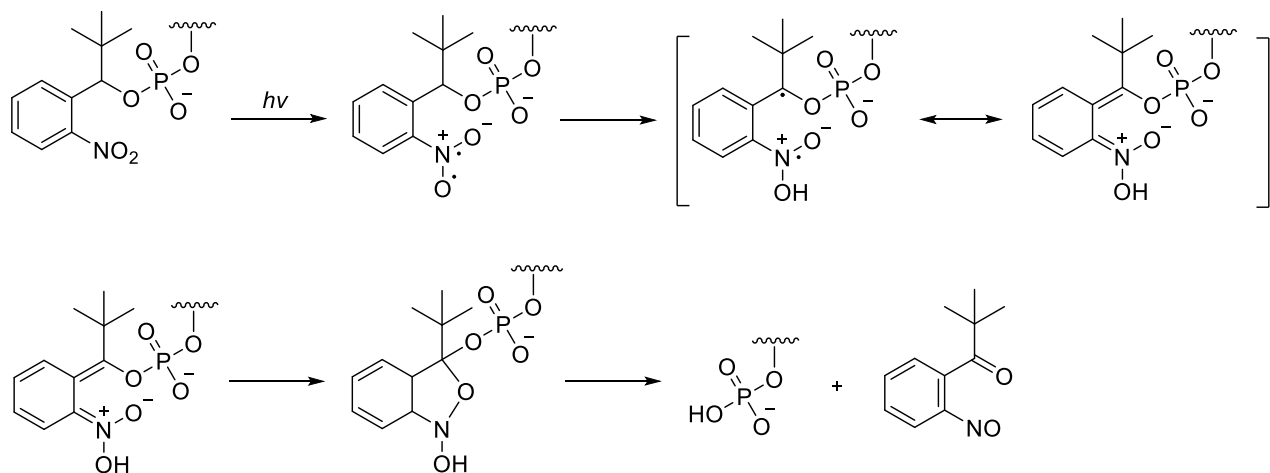
PG = Protective Group

(ii) Synthesis of 5'-monophosphorylated RNAs



PG = Protective Group
TOM = Trisopropylsilyloxymethyl

Deprotection mechanism of nitrobenzyl group

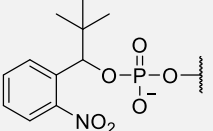
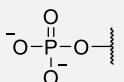


<Photo-deprotection conditions using UV irradiation>

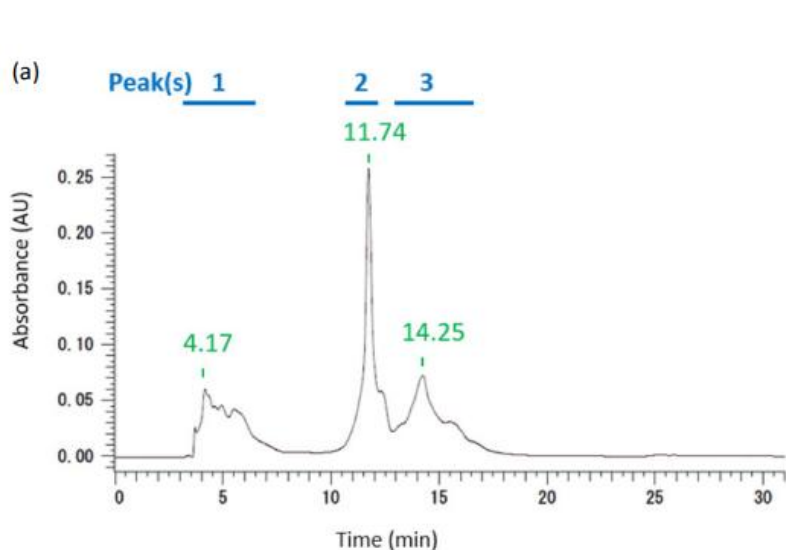
UV irradiation at a wavelength of 365 nm (intensity: 4 mW/cm²) for 10 minutes

HPLC profile and dPAGE image of the 131-nucleotide 5'-monophosphorylated RNA

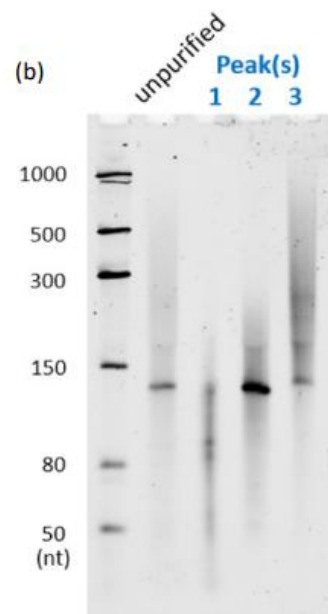
Table. List of Chemically synthesized RNA

Length	Sequence(5' → 3')*	5'-Structure	Retention Time (minutes)
131	G _m G _m AGCCACCAUGGGAUGGAGCUGUAUCAUCC UCUUCUUGGUAGCAACAGCUACAGGCGCGCACU CCAGUAUAAUCAACUUUGAAAAACUGAGCGAGA AGGACGAGCUGUAGAAAAAAAAAAAAAAAAAAAAA		15.87
			10.37

*Gm : 2'-O-Methylguanosine



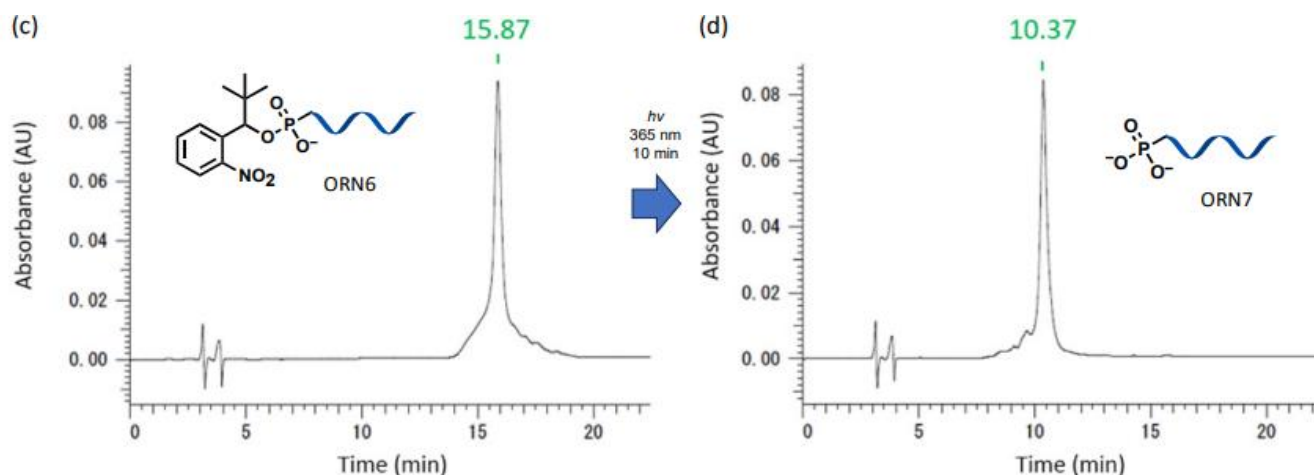
(a) HPLC profile of the crude RNA



(b) dPAGE image of peak 1-3 of crude HPLC profile (5% dPAGE containing 7M urea)

HPLC condition

Column size : C4 (250 x 4.6 10 mmI.D., S-5 μm, 30 nm)
 Mobile phase : A) 50 mM TEAA buffer (pH 7.0) + 5% CH₃CN
 B) CH₃CN
 Gradient: over 20min B=5-15%
 Flow rate : 1 mL/min
 Temperature : 50°C
 Detection: 260 nm
 Loop size: 2.0 mL



(c) HPLC profile of RNA after RP-HPLC purification

(d) HPLC profile of the produced 5'-monophosphate RNA by UV-irradiation of 5'-Nb-protected RNA

HPLC condition

Column size : C18 (250 x 4.6 mmI.D., S-5 μ m, 12 nm)
 Mobile phase : A) 50 mM TEAA buffer (pH 7.0) + 5% CH₃CN
 B) CH₃CN
 Gradient: over 20min B=5-15%
 Flow rate : 1 mL/min
 Temperature : 50°C
 Detection: 260 nm
 Loop size: 2.0 mL

Figure. HPLC profiles and dPAGE image for RNA synthesis and UV-induced deprotection.

High-Efficiency, High-Purity Separation and Purification of Long-Chain RNA more than 100 Nucleotides !

<Reference>

Ototake, M., et al.: *Nucleic Acids Research*, **52**, 12141(2024).

Listed products are intended for laboratory research use only, and not to be used for drug, food or human use. Please visit each region's website for product information. This leaflet may contain products that cannot be exported to your country due to regulations. Bulk quote requests for some products are welcomed. Please contact us.

Japan
FUJIFILM Wako Pure Chemical Corporation
 1-2, Doshomachi 3-Chome, Chuo-ku, Osaka 540-8605, Japan
 ffwk-cs@fujifilm.com
 labchem-wako.fujifilm.com

Chinese Mainland
FUJIFILM Wako (Guangzhou) Trading Corporation
 Room 3002, 3003, 3011, 30/F., Dong Shan Plaza,
 69 Xian Lie Middle Road, Yuexiu District, Guangzhou, 510095, China
 wkgz.info@fujifilm.com
 labchem.fujifilm-wako.com.cn

The Americas
FUJIFILM Biosciences
 2501 Pullman Street, Santa Ana, CA 92705, USA
 supportfilsilssupport@fujifilm.com
 fujifilmbiosciences.fujifilm.com

Hong Kong SAR
FUJIFILM Wako Chemicals (Hong Kong) Limited
 Units 9-12 and 15-18, Level 28, Tower 1, The Millennity,
 98 How Ming Street, Kwun Tong, Kowloon, Hong Kong
 wkhk.info@fujifilm.com
 labchem.fujifilm-wako.com.cn

Europe, Middle East, and Africa
FUJIFILM Wako Chemicals Europe GmbH
 Fuggerstr 12, 41468 Neuss, Germany
 labchem_wkeu@fujifilm.com
 labchem-wako.fujifilm.com

Other Areas
 fujifilm.com/ffwk/en/about/partners/labchem