

Reagents for Nucleic Acid Synthesis



Product features

- Lineup of ancillary reagents for phosphoramidite method
- Provides products with guaranteed low water content based on advanced dehydration technology
- Customizable composition and scale-up
- Labels in different colors by reagent type to avoid mix-ups

Product Line-up

- | | |
|------------------------|-------------------------|
| ● Deblocking Reagents | ● Phosphoramidites |
| ● Activators | ● Solid Supports |
| ● Capping Reagents | ● Solvents |
| ● Oxidation Reagents | ● Purification Products |
| ● Sulfurizing Reagents | ● Common Chemicals |

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Introduction, Nucleic acid synthesis

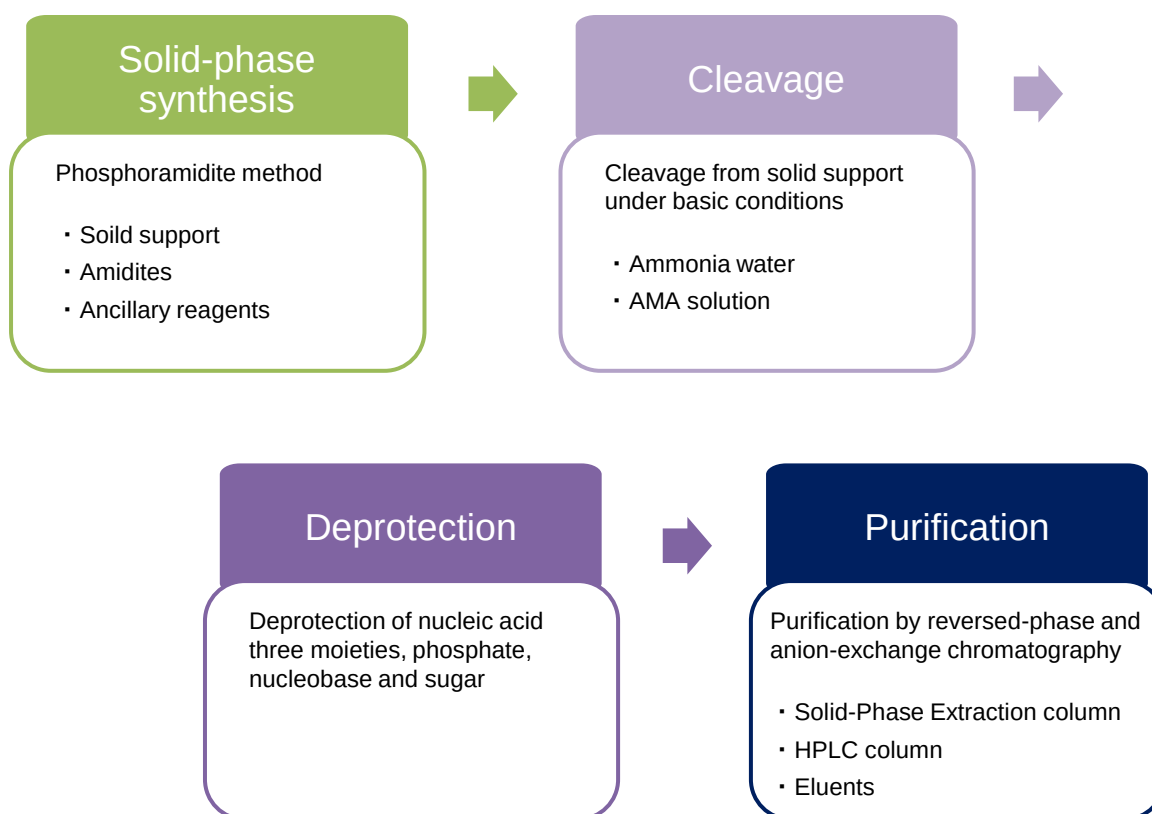
Introduction

Our lineup of nucleic acid synthesis reagents focuses on ancillary reagents and dehydration solvents for use in the synthesis of oligonucleotides using the phosphoramidite method. We provide reagents of suitable quality for nucleic acid synthesis by leveraging our unique liquid preparation, synthesis, dehydration, and analysis technologies developed over years of reagent manufacturing.

Nucleic acid

“Nucleic acids” are a collective term for deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Its constituent molecule is an oligonucleotide, a structure of bases, sugars, and phosphates linked by phosphodiester bonds. “Nucleic acid medicine,” which has attracted considerable attention in recent years, uses DNA and RNA molecules, which are the carriers of genetic information in living organisms, as drugs. There is a high expectation that this approach will lead to next-generation drugs because they can act on biological molecules that cannot be targeted by conventional small-molecule agents or antibody drugs.¹⁾ Another characteristic is that oligonucleotides, the main component of “nucleic acid drugs,” can be manufactured by organic synthesis (mainly solid-phase synthesis) using an automated synthesizer.

Synthesis of oligonucleotide by solid-phase synthesis



Nucleic Acid Synthesis Method

Phosphoramidite method

The traditional method for synthesizing oligonucleotides is called the phosphoramidite method, which is a form of solid-phase synthesis that involves the addition of a phosphoramidite monomer (monoamide of phosphite diester) to a solid-phase support. The fundamental reaction to bind the amidite, which is a nucleic acid monomer, consists of 4 steps:

Step 1 detritylation → Step 2 amidite coupling reaction → Step 3 capping reaction → Step 4 oxidation or sulfurization. A cycle consisting of these 4 steps is repeated until the desired strand length is obtained (see Figure 1). An automated synthesizer is used for this reaction.

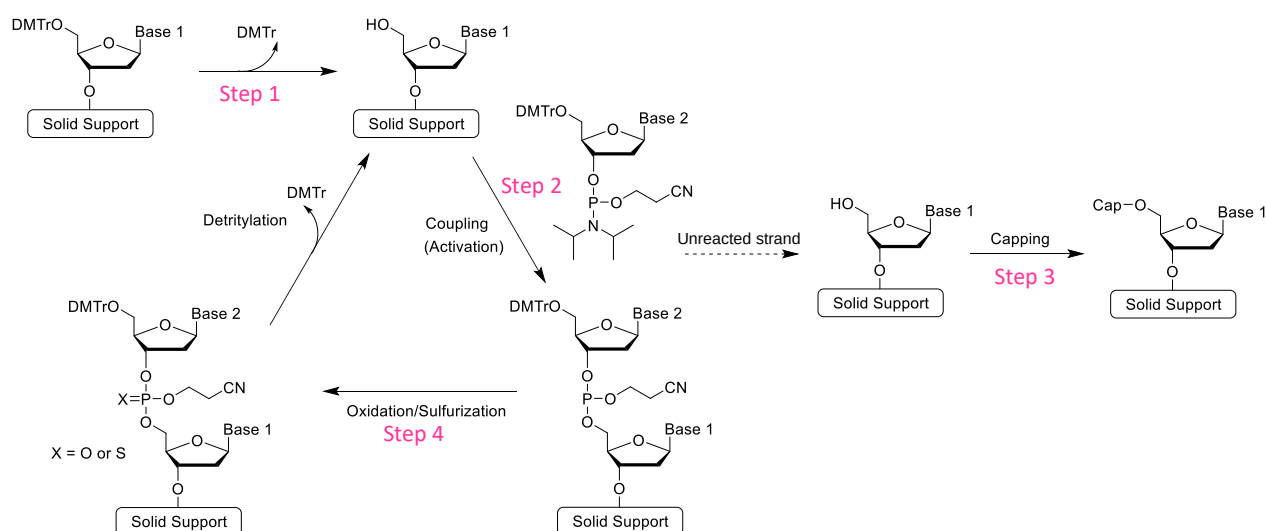
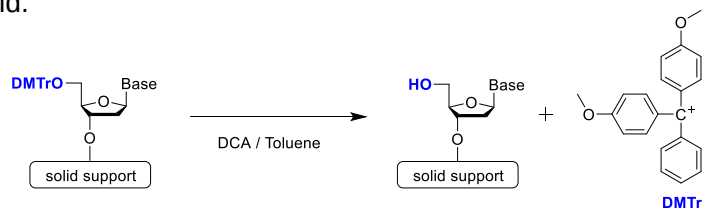


Fig 1. Reaction mechanism of phosphoramidite method

Reactions and reagents for the phosphoramidite method²⁾

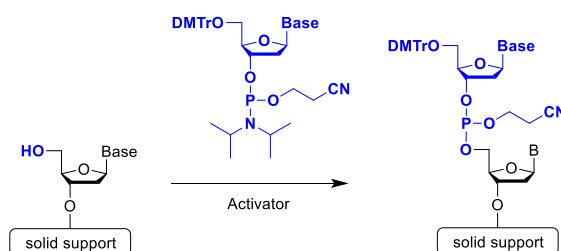
Step 1 Detritylation

Step 1 is detritylation. 4,4'-Dimethoxytrityl group (DMTr group) is often used to protect the hydroxy group at the 5' position. Since the trityl cation is stabilized by two methoxy groups, it can be easily cleaved by treatment with a weak acid. Dichloroacetic acid or trichloroacetic acid is used as the acid.



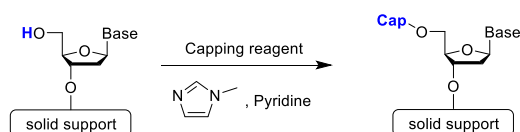
Step 2 Coupling Reaction (Activation)

Step 2 is the coupling reaction. Condensation occurs between the nucleotide unit to be bound and the nucleoside on the solid support or the oligonucleotide being synthesized. In general, 5-Benzylthio-1*H*-tetrazole (BTT), 5-Ethylthio-1*H*-tetrazole (ETT), or 4,5-Dicyanoimidazole (DCI) is used to activate phosphoramidite.



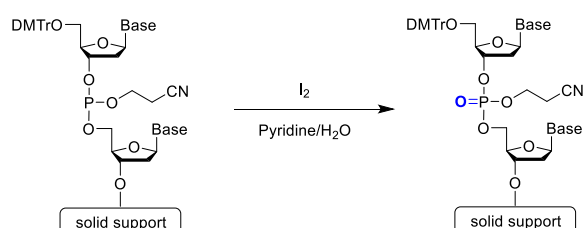
Step 3 Capping Reaction

Step 3 is the capping reaction. In the phosphoramidite method, an incomplete oligo that has one less base than the desired oligonucleotide may be formed if the coupling reaction does not progress completely and proceeds to the next step with an unreacted hydroxy group remaining. Therefore, the 5'-OH group of the unreacted strand is acetylated to prevent elongation reaction.



Step 4 Oxidation

Step 4 is oxidation. A phosphite ester bond is formed during phosphoramidite coupling. This is slightly unstable and may cause a side reaction during further elongation reactions. To prevent this, it is converted into a stable phosphate ester. Iodine (I₂) is the most commonly used oxidizing reagent.



Purification process of nucleic acid

The oligonucleotide synthesized by solid-phase synthesis is retained on the solid support and is thus cleaved using a base. The protecting groups that were introduced into reactive substituent groups during oligonucleotide synthesis cycles to avoid their participation in coupling are deprotected to complete the reaction. After cleavage and deprotection, the crude material is separated and purified by reversed-phase or anion-exchange chromatography to obtain the desired oligonucleotide.³⁾

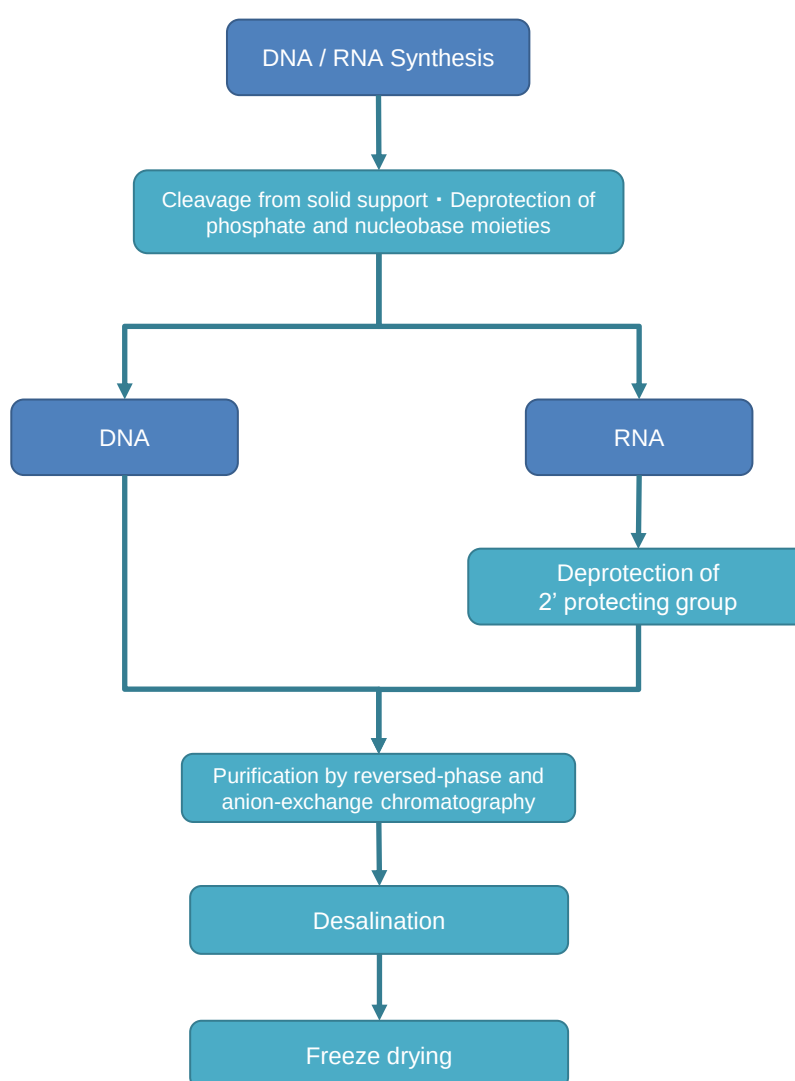


Fig 2. Separation/purification process after DNA/RNA synthesis

[References]

- 1) [2016 Edition: Current status and future prospects of global nucleic acid drug development] 2016: Sekai no kakusaniyakuhinkaiatsu no genjyo to syoraitenbou (in Japanese). Seed Planning, Inc. (2016) .
- 2) [Biotechnology reagents] Baiotekunorogi shiyaku (in Japanese). The Chemical Daily Co., Ltd. (1989).
- 3) Hirao Ichiro., Kurumizaka Hitoshi., [Principles and protocols of nucleic acid experiments that can be selected according to purpose] Mokutekibetsu de eraberu kakusanjikken no genri to purotokoru (in Japanese). Yodosha Company, Ltd. (2011).

Ancillary reagents

Features

- Lineup of ancillary reagents for phosphoramidite method
- Provides products guaranteed to have a low water content based on advanced dehydration technology
- Supports customization of concentration and composition
- Supports scale-up and mass production



Fig 3. Reagent bottles and label colors.

Labels of different colors used for each reagent type to avoid mix-ups



Deblocking Reagents



Activators



Capping Reagents
(Cap A)



Capping Reagents
(Cap B)



Oxidation Reagents



Sulfurizing Reagents

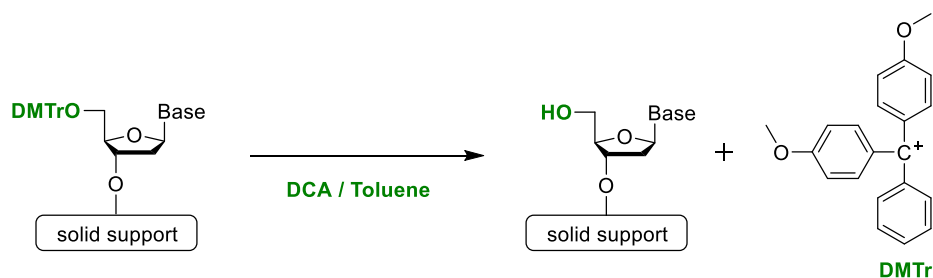


Cap connection between reagent bottles and automated synthesizers

- The glass gallon container used for our 3 L packaging does not fit the bottle cap provided with ÄKTA oligopilot. Before use, transfer it to a container with the GL45 screw thread.



Deblocking Reagents



Toluene

Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
043-34441	Deblocking Solution [Dichloroacetic Acid-Toluene (3:97)]	3 L	≤200ppm	Keep at RT

DCM

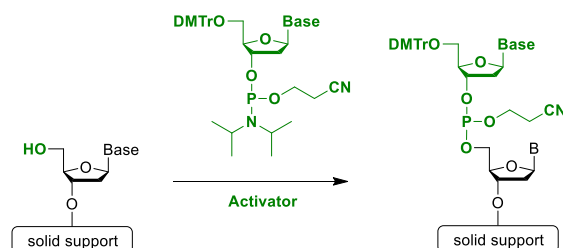
Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
048-28923	Deblocking Solution-1 (3w/v% Trichloroacetic Acid, Dichloromethane Solution)	1 L	≤40ppm	Keep at RT
042-28921		3 L		
042-28926		3 L×4		

• For preparation at the time of use, use the following reagent

Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
200-02402	Trichloroacetic Acid	25 g	—	Keep at RT
202-02401		100 g		
204-02405		500 g		
042-31231	Dichloromethane, Super Dehydrated	100 mL	≤10 ppm	Keep at RT
044-31235		500 mL		
048-31233		3 L		
040-16653	Dichloroacetic Acid	25 mL	—	Keep at RT
044-16656		500 mL		
202-17911	Toluene, Super Dehydrated	100 mL	≤10 ppm	Keep at RT
204-17915		500 mL		
206-17914		3 L		



Activators



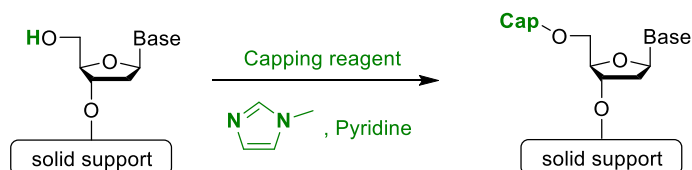
Powder / Solid

Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Water Content	Storage Condition
029-19862	5-Benzylthio-1 <i>H</i> -tetrazole		21871-47-6	25 g	≤ 600 ppm	Keep at 2-10 degrees C.
023-19865				500 g		
044-34851	4,5-Dicyanoimidazole		1122-28-7	5 g	≤ 300 ppm	Keep at RT
042-34852				25 g		
046-34855				500 g		
051-09491	5-Ethylthio-1 <i>H</i> -tetrazole		89797-68-2	5 g	≤ 300 ppm	Keep at RT
059-09492				25 g		
053-09495				500 g		
130-19221	<i>N</i> -Methylbenzimidazolium Trifluoromethanesulfonate		361447-89-4	5 g	≤ 300 ppm	Keep at RT
138-19222				25 g		
161-29041	<i>N</i> -(Phenyl)imidazolium Trifluoromethanesulfonate		361447-81-6	5 g	≤ 300 ppm	Keep at RT
169-29042				25 g		

Solution

Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Water Content	Storage Condition
013-19685	Activator Solution-1 (0.25 mol/L 4,5-Dicyanoimidazole, Acetonitrile Solution)		1122-28-7	500 mL	≤ 30 ppm	Keep at RT
011-19681				3 L		
013-19705	Activator Solution-2 (0.45 mol/L 1 <i>H</i> -Tetrazole, Acetonitrile Solution)		288-94-8	500 mL	≤ 30 ppm	Keep at RT
011-19701				3 L		
017-20014	Activator Solution-3 (0.25 mol/L 5-Benzylthio-1 <i>H</i> -tetrazole, Acetonitrile Solution)		21871-47-6	100 mL	≤ 30 ppm	Keep at RT
015-20015				500 mL		
013-20011				3 L		
010-19695	Activator Solution-4 (0.25 mol/L 5-Ethylthio-1 <i>H</i> -tetrazole, Acetonitrile Solution)		89797-68-2	500 mL	≤ 30 ppm	Keep at RT
018-19691				3 L		
012-19694				3 L×4		
013-28735	Activator Solution (0.5mol/L 5-Ethylthio-1 <i>H</i> -tetrazole, Acetonitrile Solution)		89797-68-2	500 mL	≤ 30 ppm	Keep at RT

Capping Reagents



THF

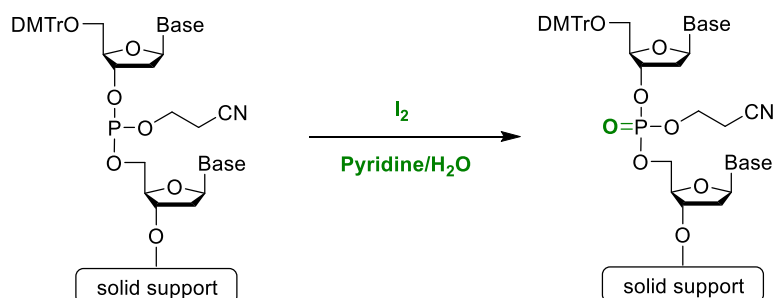
Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
038-18995	Cap A Solution-1 (10vol% Acetic anhydride/Tetrahydrofuran Solution)	500 mL	≤100ppm	Keep at RT
036-18991		3 L		
032-18993		3 L×4		
035-19005	Cap B Solution-1 [Tetrahydrofuran/1-Methylimidazole/Pyridine (8:1:1) Solution]	500 mL	≤100ppm	Keep at RT
033-19001		3 L		
039-19003		3 L×4		
030-19016	Cap A Solution-2 [Tetrahydrofuran/Acetic Anhydride/Pyridine (8:1:1) Solution]	500 mL	≤100ppm	Keep at RT
030-19011		3 L		
034-19014		3 L×4		
037-19026	Cap B Solution-2 (10vol% 1-Methylimidazole/Tetrahydrofuran Solution)	500 mL	≤100ppm	Keep at RT
037-19021		3 L		
031-19024		3 L×4		

Acetonitrile

Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
036-25385	Cap B1 Solution [Acetic Anhydride-Acetonitrile (4:6)]	500 mL	—	Keep at RT
034-25381		3 L		
039-25635	Cap B2 Solution [Pyridine-Acetonitrile (6:4)]	500 mL	≤100ppm	Keep at RT
033-25633		3 L		
039-25375	Cap B2 Solution [2,6-Lutidine-Acetonitrile (6:4)]	500 mL	≤100ppm	Keep at RT
031-25374		2.5 L		
037-25371		3 L		
034-25401	Cap B Solution [Acetic Anhydride-2,6-Lutidine-Acetonitrile (2:3:5)]	3 L	—	Keep at RT



Oxidation Reagents



THF

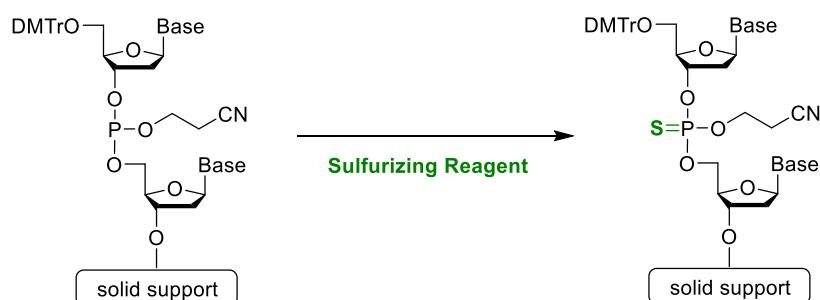
Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
150-03635	Oxidizing Solution [Iodine Solution (abt. 0.02mol/L)][Tetrahydrofuran:Pyridine:Water(78:20:2)]	500 mL	-	Keep at RT
158-03631		3 L		
158-02455	Oxidizing Solution-2 [0.1 mol/L I_2 • THF:Pyridine:Water(78:20:2)Solution]	500 mL	—	Keep at RT
156-02451		3 L		
152-02453		3 L×4		

THF free

Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
150-03515	Oxidizing Solution [Iodine Solution (abt. 0.05 mol/L)][Pyridine:Water(9:1)]	500 mL	—	Keep at RT
158-03516		2.5 L		

Sulfurizing Reagents

Chemical modifications of the nucleic acid phosphate moiety include phosphorothioate modification where O (oxygen atom) is substituted by S (sulfur atom). In nucleic acid medicine, sulfurizing modification is used mainly for antisense drugs.¹⁾ Phosphorothioate synthesis is achieved by having a sulfurizing reagent act on the phosphite ester formed by a coupling reaction.



Powder / Solid

Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Water Content	Storage Condition
012-28582	5-Amino-3 <i>H</i> -1,2,4-dithiazole-3-thione		6846-35-1	25 g	≤ 5000 ppm	Keep below 25 degrees C.
014-28581				100 g		
016-28585				500 g		
027-19422	Bis(phenylacetyl) Disulfide 【PADS】		15088-78-5	25 g	—	Keep at RT
029-19421				100 g		
021-19425				500 g		
166-28251	5-Phenyl-3 <i>H</i> -1,2,4-dithiazol-3-one		7047-10-1	5 g	≤200ppm	Keep at 2-10 degrees C.
164-28252				25 g		
168-28255				500 g		



The most commonly used reagent, DDTT, is highly crystalline and hardly dissolves in acetonitrile, the reaction solvent. Therefore, acetonitrile is spiked with pyridine to increase its solubility. On the other hand, 5-phenyl-3*H*-1,2,4-dithiazole-3-one shows favorable solubility in acetonitrile and is thus a convenient sulfurizing reagent that can be used pyridine-free.

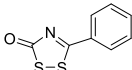
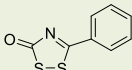
[Reference]

1) Takao Inoue., : *Drug Delivery System.*, 31-1, **10** (2016). (in Japanese)



Sulfurizing Reagents

Solution

Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Water Content	Storage Condition
199-18751	Sulfurizing Solution (0.05 mol/L 5-Phenyl-3 <i>H</i> -1,2,4-dithiazol-3-one, Acetonitrile Solution)		7047-10-1	100 mL	—	Keep at RT
191-18755				500 mL		
192-18741	Sulfurizing Solution (0.1 mol/L 5-Phenyl-3 <i>H</i> -1,2,4-dithiazol-3-one, Acetonitrile Solution)		7047-10-1	100 mL	—	Keep at RT
194-18745				500 mL		

Our sulfurizing solutions are distributed after the stability of the compound in organic solvents is confirmed.

[Notes]

Sulfurizing reagents manufactured by FUJIFILM Wako Pure Chemical Corporation or FUJIFILM Wako Chemical Corporation are distributed for research-only purposes. If you are seeking products for commercial use, please refer to the related patents of the compound.

Reagents for Oligopilot

Features

- Composition for automated synthesizer ÄKTA oligopilot (Cytiva)
- Free from THF which may cause deterioration of the synthesizer flow channel
- Can be supplied from laboratory to industrial scale



Fig 4. ÄKTA oligopilot

Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
Deblocking Reagents				
043-34441	Deblocking Solution [Dichloroacetic Acid-Toluene (3:97)]	3 L	≤200ppm	Keep at RT
Activators				
011-19681	Activator Solution-1 (0.25 mol/L 4,5-Dicyanoimidazole, Acetonitrile Solution)	3 L	≤30ppm	Keep at RT
011-19701	Activator Soluiton-2 (0.45 mol/L 1 <i>H</i> -Tetrazole, Acetonitrile Solution)	3 L	≤30ppm	Keep at RT
013-20011	Activator Solution-3 (0.25 mol/L 5-Benzylthio-1 <i>H</i> -tetrazole, Acetonitrile Solution)	3 L	≤30ppm	Keep at RT
018-19691	Activator Solution-4	3 L	≤30ppm	Keep at RT
012-19694	(0.25 mol/L 5-Ethylthio-1 <i>H</i> -tetrazole, Acetonitrile Solution)	3 L×4		
Capping Reagents				
034-25381	Cap B1 Solution [Acetic Anhydride-Acetonitrile (4:6)]	3 L	—	Keep at RT
033-25633	Cap B2 Solution [Pyridine-Acetonitrile (6:4)]	3 L	≤100ppm	Keep at RT
037-25371	Cap B2 Solution [2,6-Lutidine-Acetonitrile (6:4)]	3 L	≤100ppm	Keep at RT
034-25401	Cap B Solution [Acetic Anhydride-2,6-Lutidine-Acetonitrile (2:3:5)]	3 L	—	Keep at RT
Oxidation Reagents				
158-03516	Oxidizing Solution [Iodine Solution (abt. 0.05mol/L)][Pyridine:Water(9:1)]	2.5 L	—	Keep at RT

Phosphoramidites

Provides high-quality phosphoramidite

Features

- Supports small scale to bulk scale
- Supplies company-specific products in containers compatible with each synthesizer, such as ABI, Expedite, MerMade, and ÄKTA oligopilot

Modified phosphoramidite

Nucleic acid drugs have chemical modifications of the nucleobase, phosphate, or sugar moiety for functional improvements, such as acquisition of nuclease resistance and improvement of binding affinity to target nucleic acids. Modifications of the sugar moiety include 2'-position modifications and bridging modifications.

2'-Fluoro (2'-F), 2'-O-Methyl (2'-O-Me), and 2'-O-Methoxyethyl (2'-O-MOE) modifications are known 2'-position modifications. Kynamro[®], a nucleic acid drug marketed in 2013, is a representative Gapmer-type antisense, which uses 2'-MOE at both ends (wing sites) of the oligo nucleic acid to increase binding affinity to the target RNA in addition to sulfurization to improve in vivo stability.¹⁾²⁾

Bridging modifications were developed under the concept of "fixing the fluctuating conformation of the sugar moiety by bridging." The sugar moiety can be fixed to the RNA type (N type) by chemically modifying the 2'- and 4'-positions of the sugar moiety. This confers excellent binding affinity to the target complementary strand nucleic acid, and furthermore, steric hindrance due to bridging is expected to improve functionality including nuclease resistance. In 1997, 2',4'-BNA/LNA (bridged nucleic acid) was developed by Imanishi, Obika, and others from the School of Pharmaceutical Sciences, Osaka University.¹⁾²⁾

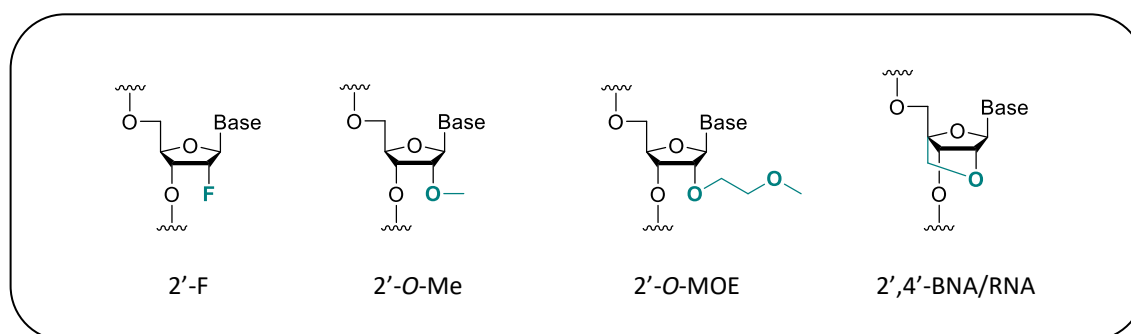


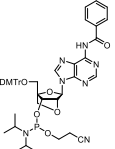
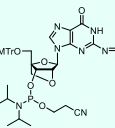
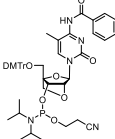
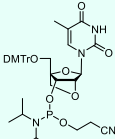
Fig 5. Structure of modified nucleic acids

[References]

1) Takao Inoue., : *Drug Delivery System.*, 31-1, **10** (2016). (in Japanese)

2) "Development and Applications of Nucleic Acid Therapeutics" ed. by Takeshi Wada., CMC Publishing Co.,Ltd. (2016). (in Japanese)

Locked Nucleic Acid

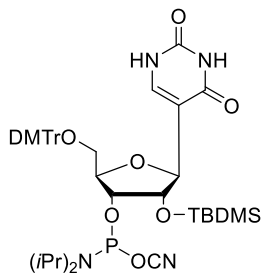
Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Water Content	Storage Condition
128-06771	Locked Nucleic Acid-A(Bz) Cyanoethyl Phosphoramidite (mixture of isomers)		206055-79-0	1 g	≤0.5%	Keep at -20 degrees C.
124-06773				5 g		
125-06781	Locked Nucleic Acid-G(DMF) Cyanoethyl Phosphoramidite (mixture of isomers)		709641-79-2	1 g	≤0.5%	Keep at -20 degrees C.
121-06783				5 g		
122-06791	Locked Nucleic Acid-mC(Bz) Cyanoethyl Phosphoramidite (mixture of isomers)		206055-82-5	1 g	≤0.5%	Keep at -20 degrees C.
128-06793				5 g		
125-06801	Locked Nucleic Acid-T Cyanoethyl Phosphoramidite (mixture of isomers)		206055-75-6	1 g	≤0.5%	Keep at -20 degrees C.
121-06803				5 g		

We supply locked nucleic acids in containers (glass bottles) that meet our specifications.

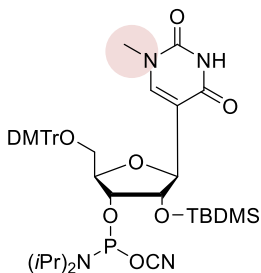
Modified phosphoramidite

I Pseudouridine

Pseudouridine, an isomer of uridine, is one of the most common post-transcriptional modifications found in RNA. Whereas uridine forms a C–N glycosidic bond with the ribose ring, pseudouridine forms a C–C bond. This structural difference confers distinct properties and functions to pseudouridine-containing RNA across a wide range of biological processes. Pseudouridine is particularly important for regulating RNA stability and function, and it plays a critical role in the development of RNA therapeutics, especially mRNA vaccines. In COVID-19 mRNA vaccines, pseudouridine played a significant role in suppressing innate immune responses, improving RNA stability, and increasing translational efficiency.



DMT-2'-O-TBDMS-Pseudouridine (ψ)



DMT-2'-O-TBDMS- N^1 -Methylpseudouridine ($m^1\psi$)

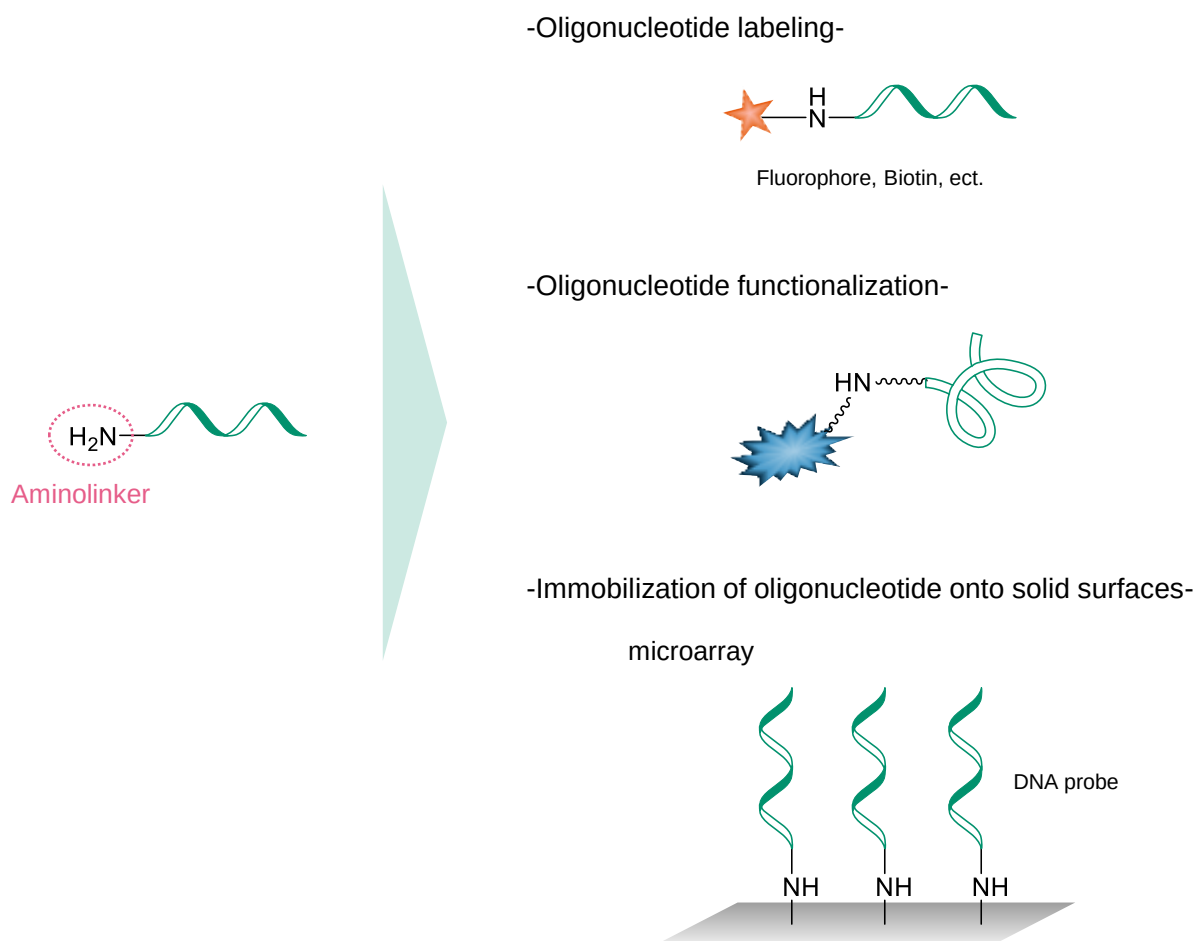
Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Water Content	Storage Condition
046-35151	DMT-2'-O-TBDMS-pseudouridine Phosphoramidite (mixture of isomers)		163496-23-9	250 mg	≤0.1%	Keep at -20 degrees C.
043-35161	DMT-2'-O-TBDMS- N^1 -methyl pseudouridine Phosphoramidite (mixture of isomers)		875302-45-7	250 mg	≤0.1%	Keep at -20 degrees C.

We supply pseudouridine amidites in containers (glass bottles) that meet our specifications.

Aminolinkers (amino-modifying reagents)

I Amino Linker

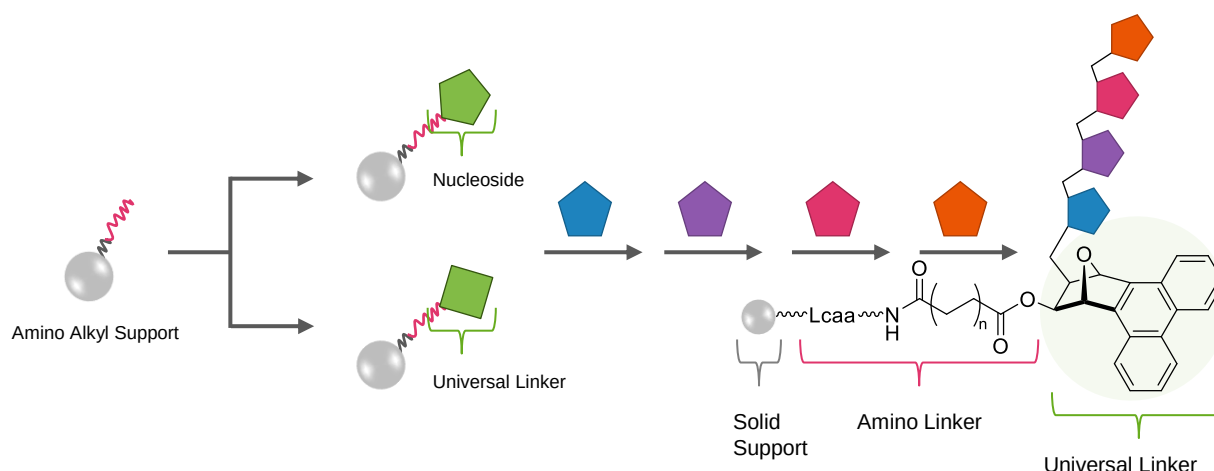
<Usage>



Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Water Content	Storage Condition
135-19651	MMT-C6-Amino Linker		114616-27-2	250 mg	≤0.1%	Keep at -20 degrees C.
194-19421	ssH-Linker		922522-12-1	250 mg	≤0.1%	Keep at -20 degrees C.

Solid Supports

Oligonucleotides are chemically synthesized using an automated synthesizer based on the solid-phase synthesis method. In this method, a nucleoside molecule or a universal linker is anchored via a spacer to an amino-functionalized solid support at the 3' terminus. The support is then placed in a reaction vessel, and repeated synthesis cycles are carried out to elongate the oligonucleotide chain.



Solid supports used for oligonucleotide synthesis include controlled pore glass (CPG) and cross-linked polystyrene (non-swelling polystyrene and swelling polystyrene). Non-swelling supports offer advantages such as high coupling efficiency, resulting in high synthetic purity, as well as ease of washing. Because of their lower linker loading, they are suitable for small-scale synthesis and have recently been applied to long-chain nucleic acid synthesis. In contrast, swelling supports allow for high loading capacity, enabling high-yield synthesis, and are used for the commercial production of nucleic acid therapeutics. Thus, selecting the appropriate support according to the required nucleic acid quality, synthesis scale, and chain length is essential in oligonucleotide synthesis¹⁾.

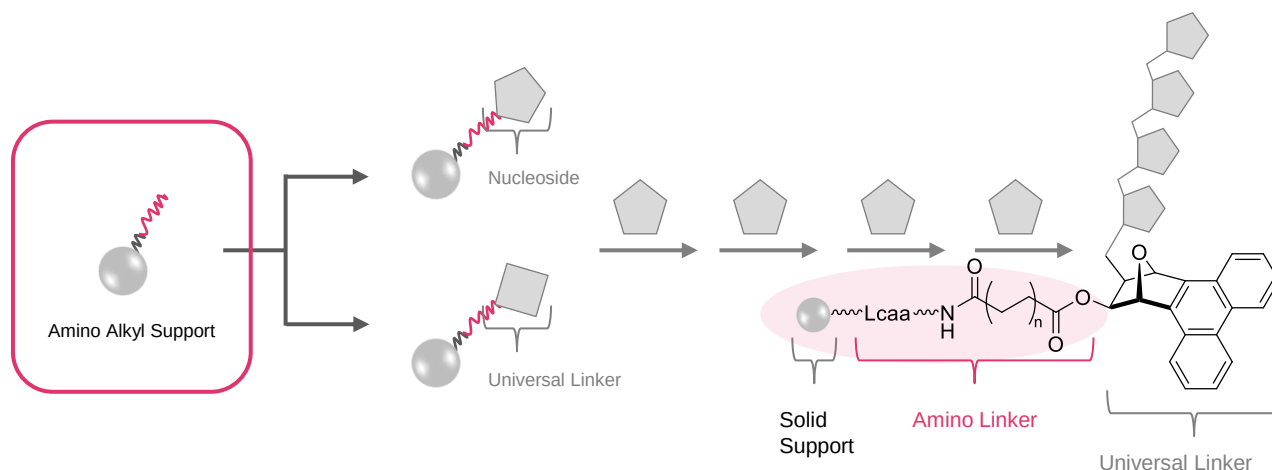
Comparison of Support Properties

	Controlled pore glass	Highly Cross-linked Polystyrene	Low Cross-linked Polystyrene
Linker loading	Low – Medium	Low	High
Synthesis scale	Small	Small	Large
Oligonucleotide chain length	Short – Long	Short – Long	Short
Selection of pore size	500 - 4000 Å	500 – 4000 Å	Not controlled
Swelling property	None	None	Present
Washing efficiency	High	High	Moderate
Surface property	Hydrophilic	Hydrophobic	Hydrophobic
Suitability for long nucleic acids	Suitable	Suitable	Not suitable

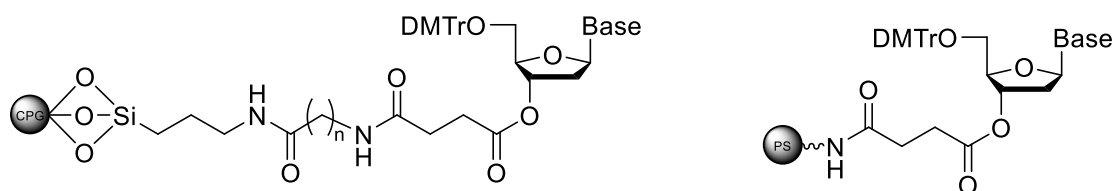
[References]

- 1) Mitsuo Sekine., : *Journal of The Surface Finishing Society of Japan*, 68(8), 443 (2017). (in Japanese)

Amino Alkyl Supports



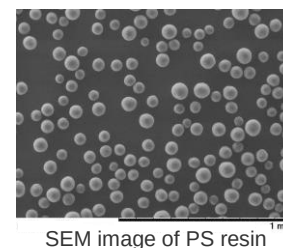
<Structures of Representative Nucleoside-Linked Solid Supports>



■ PS Support | Highly Cross-linked Polystyrene

Features

- Solid support for chemical synthesis of oligonucleotides
- Porous, non-swelling polystyrene support
- Precisely controlled physical characteristics such as particle size, shape, and pore size of the polystyrene resin



Structure	Code No.	Support	Pore size	Reactive group	Amino Loading	Pkg. Size
	018-28981	PS	1000 Å	Amino	40-60 $\mu\text{mol/g}$	1 g
	—	PS	2000 Å	Amino	—	1 g
	—	PS	3000 Å	Amino	—	1 g

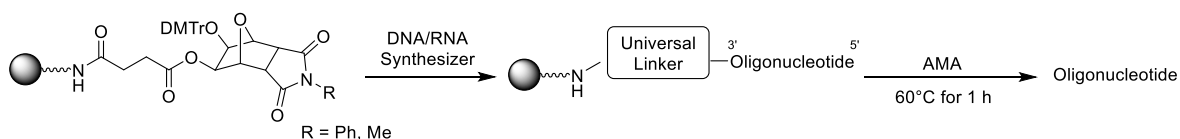
Bulk quantities and custom modifications tailored to your specifications are also available. Please contact us for further details.

<Physical Properties>

	Aminolinker PS 1000A
Particle size	53-100 μm
Pore size	1000 Å (100 nm)
Dry volume	3.9 mL/g
Swelling volume in acetonitrile	4.1 mL/g
Swelling volume in toluene	4.1 mL/g
Swelling volume in THF	—
Swelling volume in dichloromethane	—
Reactive group	NH ₂
Amino loading	40-60 $\mu\text{mol/g}$

<Application>

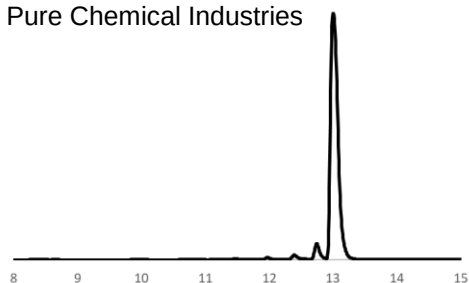
■ Synthesis of Oligonucleotides (T₁₀) Using Aminolinker PS 1000A in DMTr-Off Mode



Solid support				UnyLinker™		Results
	Supplier	Beads	Pore size	R	Compound loading	Area ratio of T ₁₀
(a)	Wako	PS	1000Å	Ph	55 μmol/g	92%
(b)	Competitor	CPG	1000Å	Me	45 μmol/g	92%

Sequence : 5'-TTT TTT TTT T-3'

a) PS 1000 Å from FUJIFILM Wako
Pure Chemical Industries



b) CPG 1000 Å from other company

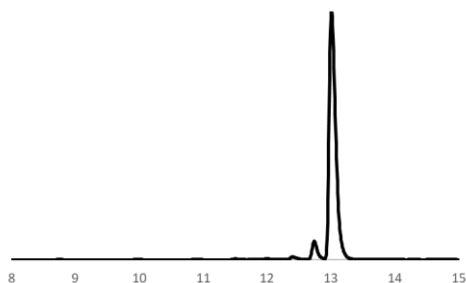


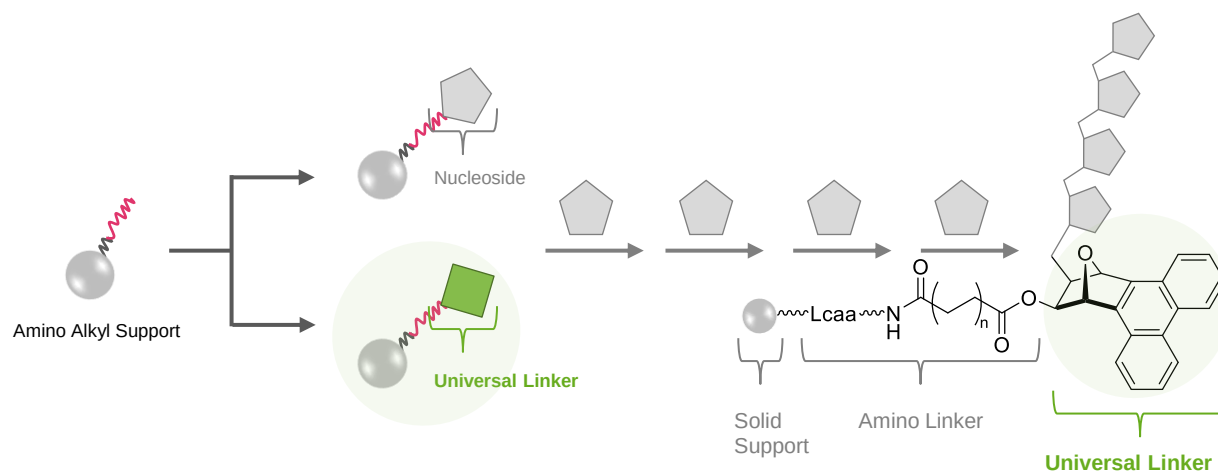
Figure HPLC analysis of ONs released from T10-loaded (a) UnyLinker™ PS 1000Å (R=Ph) and (b) UnyLinker™ CPG 1000Å (R=Me) under Basic conditions. Basic condition: AMA at 60°C for 1 h.

HPLC Condition of Oligonucleotide

Column size : Wakopak® Ultra C18-2 ϕ 2.1 mm×100 mm (D)
 Mobile phase : A) 100 mmol/L TEAA aq., B) Acetonitrile
 Gradient : 0-15 min B=5-15%, 15-20 min B=100%, 20-25 min B=5%
 Flow rate : 0.3 mL/min
 Temperature : 40°C
 Detection : UV260 nm
 Injection vol. : 0.5 μL
 Sample : DNA dT 10 mer, All PO

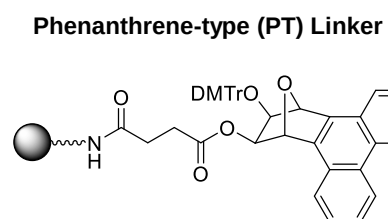
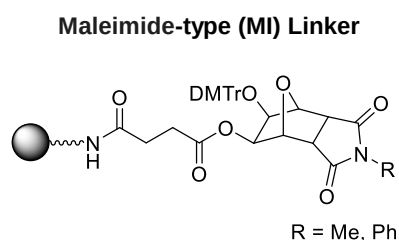
Universal Linker Supports

In solid-phase synthesis using the phosphoramidite method, the nucleoside that will constitute the 3' terminus of the oligonucleotide to be synthesized is immobilized onto a solid support via a linker. Because the 3'-terminal residue depends on the sequence of the oligonucleotide to be synthesized, this approach requires multiple solid supports: eight types loaded with DNA (dA, dG, dC, dT) and RNA (rA, rG, rC, rU) nucleosides, as well as additional supports loaded with the corresponding modified nucleosides when modified nucleic acids are to be incorporated. This makes the process labor-intensive. To address this issue, solid supports loaded with universal linkers have been developed as an alternative to conventional nucleoside linkers.



The most widely used universal linker is currently UnyLinker™, developed by Isis Pharmaceuticals, Inc. (now Ionis Pharmaceuticals, Inc.) in the United States, which features a maleimide ring. More recently, novel universal linkers with different core structures, as well as new cleavage conditions for releasing the universal linker from the solid support, have also been developed.

<Structures of Representative Universal Linkers>



PT Linker

The Phenanthrene-type Linker (PT Linker) is a universal linker for oligonucleotide synthesis. This linker consists of a bicyclic 1,2-diol structure containing a phenanthrene moiety and a succinyl unit that connects the solid support to the phenanthrene via an amide bond. Its high lipophilicity and UV-detectable phenanthrene scaffold facilitate easy separation of the targeted oligonucleotide from linker-derived impurities.¹⁾

Features

- Easy detection and separation of the targeted product from PT-derived components
- Usable under the same reaction conditions as conventional universal linkers
- Prevents ring-opening reactions typical of maleimide (MI)-type universal linkers



Structure	Code No.	Support	Pore size	Reactive group	Amino Loading	Pkg. Size
	169-29721	CPG	1000 Å	PT Linker	10-100μmol/g	1 g
	169-29841	PS	1000 Å	PT Linker	30-50μmol/g	1 g

<Oligonucleotide Synthesis in DMTr-off Mode>

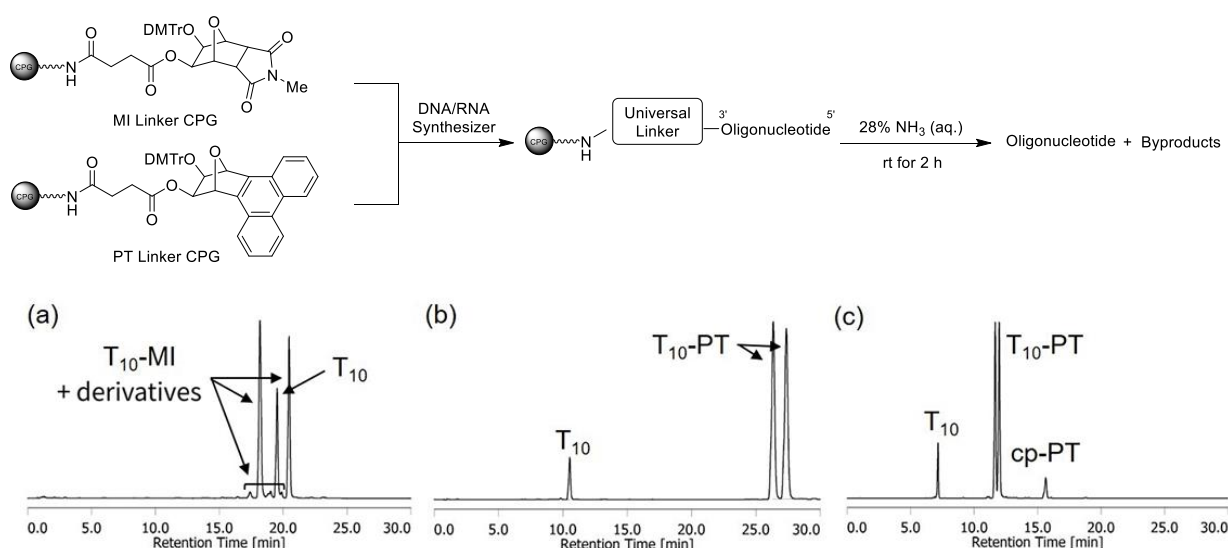


Figure 1 HPLC analysis of ONs released from T₁₀-loaded (a) MI Linker CPG (R=Me) and (b)(c) PT Linker CPG after treatment with 28% NH₃ (aq.) at rt for 2 h. HPLC analytical conditions: (a) 5-15% (b) 8-18% (c) 5-50% Acetonitrile in 0.1 M TEAA (pH 7.0) over a linear gradient for 30 min.

[References]

- 1) Yasufumi Fuchi, Kazuki Yamamoto, Yuta Ito, Yoshiyuki Hari, : *Synthesis*, **55**, 1112 (2023).

Dehydrated solvents

Acetonitrile (Low moisture guaranteed)

Features

- Water content ≤ 10 ppm guaranteed, optimal for nucleic acid synthesis due to minimal water content
- Absorbance guaranteed (260 nm, 280 nm, 400 nm)
- Can be supplied from laboratory to industrial scale

Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
017-27111	Acetonitrile, Super Dehydrated	3 L	≤ 10 ppm	Keep at RT
015-27117		18 L		

[Notes]

1. Acetonitrile is available in 3 L glass bottle and returnable container for larger volumes.
2. Returnable containers made 304 stainless steel. Coupler size is 18 L (2P on gas side, 3P on liquid side), 100L (3P on gas side, 4P on liquid side).
3. Please use up the reagents completely and return it to the agency immediately after use.



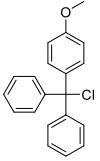
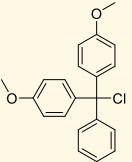
Fig 6. Image of container
(left : 3L glass bottle, center / right : stainless containers)

In addition to acetonitrile, we provide solvents with guaranteed low water content for organic synthesis.

Code No.	Product Name	Pkg. Size	Water Content	Storage Condition
167-18455	Pyridine, Dehydrated	500 mL	≤ 50 ppm	Keep at RT
165-18451		3 L		
204-17915	Toluene, Super Dehydrated	500 mL	≤ 10 ppm	Keep at RT
206-17914		3 L		

Reagents for amidite synthesis

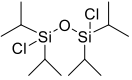
Tritylation Reagents

Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Storage Condition
130-19282	4-Methoxytrityl Chloride		14470-28-1	25 g	Keep at RT
132-19281				100 g	
046-34872	4,4'-Dimethoxytrityl Chloride		40615-36-9	25 g	Keep at RT
048-34871				100 g	

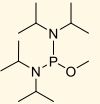
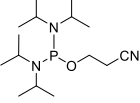
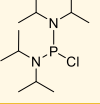
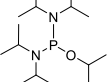
Base Reagents

Code No.	Product Name	Pkg. Size	Storage Condition
059-05352	<i>N</i> -Ethyl-diisopropylamine 【DIPEA】	25 mL	Keep at RT
164-05312	Pyridine	25 mL	Keep at RT

Silylation Reagents

Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Storage Condition
043-34681	1,3-Dichloro-1,1,3,3-tetraisopropylidisiloxane		69304-37-6	5 g	Keep at RT
041-34682				25 g	

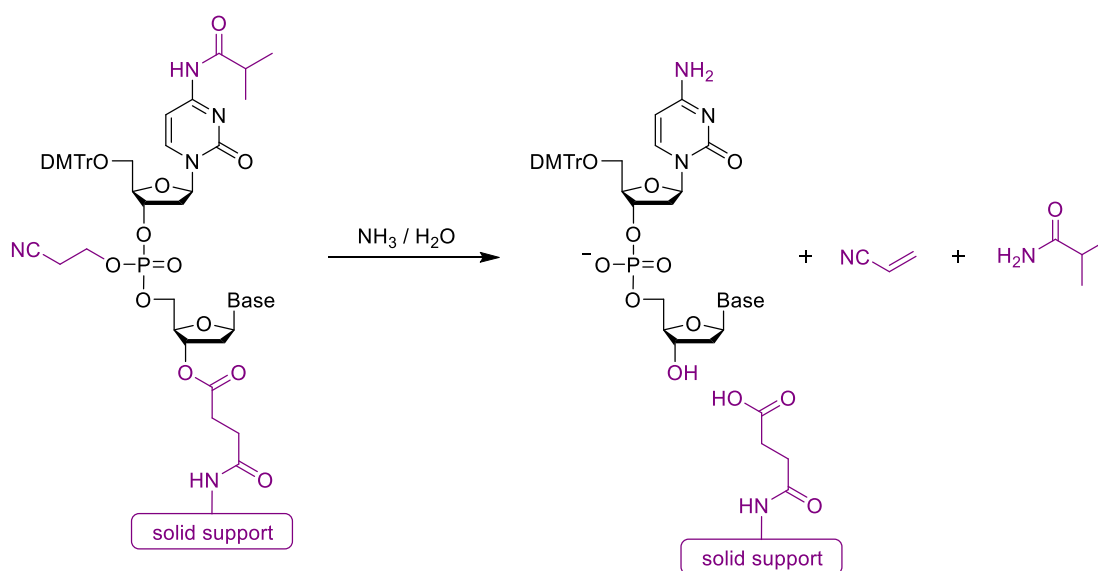
Phosphorylation Reagents

Code No.	Product Name	Structure	CAS RN®	Pkg. Size	Storage Condition
028-19751	Bis(diisopropylamino) chlorophosphine		56183-63-2	5 g	Keep at 2-10 degrees C.
026-19752				25 g	
035-25671	2-Cyanoethyl <i>N,N,N',N'</i> -Tetraisopropyl phosphordiamidite		102691-36-1	5 g	Keep at -20 degrees C.
139-19671	Methyl <i>N,N,N',N'</i> -Tetraisopropylphosphorodiamidite		92611-10-4	5g	Keep at 2-10 degrees C.
096-07821	Isopropyl <i>N,N,N',N'</i> -Tetraisopropylphosphorodiamidite		153922-24-8	5g	Keep at 2-10 degrees C.

Cleavage / Deprotection Reagents

Cleavage/Deprotection of nucleobase moieties

The crude oligonucleotide obtained by solid-phase synthesis remains bound to the solid support. After synthesis, it is cleaved from the solid support by ester hydrolysis with a base such as concentrated ammonia water. At the same time, the protecting group introduced into the amino group of the nucleobase moiety is removed under basic conditions. Instead of concentrated ammonia water, methylamine solution, AMA solution (a mixture of concentrated ammonia water and methylamine solution), or potassium carbonate/methanol solution may be used for this reaction depending on the type of protecting group.

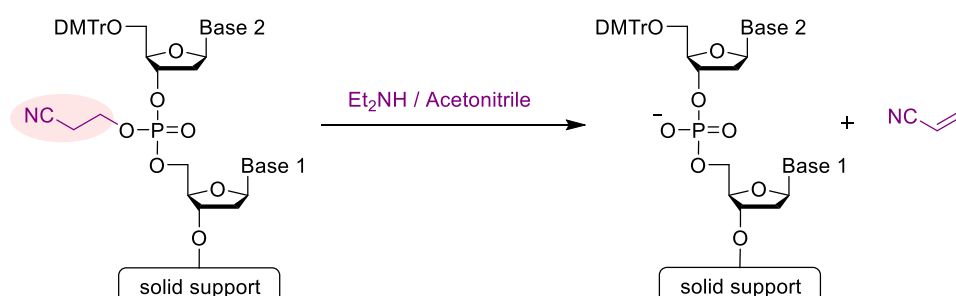


Code No.	Product Name	Pkg. Size	Storage Condition
017-03176	25% Ammonia Solution	500 mL	Keep at RT
132-01856	40% Methylamine Solution	500 mL	Keep at RT

Deprotection of phosphate moieties

Deprotection of 2-cyanoethyl group

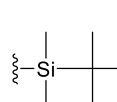
The 2-cyanoethyl group, which protects the phosphate moiety, generates acrylonitrile as a by-product when deprotected with concentrated ammonia water. Acrylonitrile may cause 2-cyanoethylation through addition reaction to the nucleobase moiety. As a method to avoid the side reaction of acrylonitrile, a weak base reagent such as diethylamine/acetonitrile solution, instead of generally used concentrated ammonia water, can be used to deprotect the 2-cyanoethyl group while maintaining binding with the solid support, followed by cleavage from the solid support and deprotection of the protecting group of the nucleobase moiety.



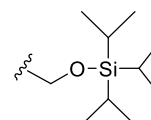
Code No.	Product Name	Pkg. Size	Storage Condition
045-34825	20% Diethylamine Acetonitrile Solution	500 mL	Keep at RT
017-27111	Acetonitrile, Super Dehydrated	3 L	Keep at RT

Deprotection of 2' protecting group

For the purpose of RNA synthesis, the hydroxy group at the 2'-position of the sugar moiety is also protected. A *tert*-butyldimethylsilyl group (TBDMS group) or triisopropylsilyloxymethyl group (TOM group) is commonly used as the protecting group. Both TBDMS and TOM groups can be deprotected by treatment with fluoride ions such as tetrabutylammonium (TBAF) solution.¹⁾



TBDMS group



TOM group

Code No.	Product Name	Pkg. Size	Storage Condition
208-20201	Tetrabutylammonium Fluoride, Tetrahydrofuran Solution (ca. 1 mol/L) 【TBAF】	100 mL	Keep at 2-10 degrees C.
200-20205		500 mL	
352-34922	Triethylamine Trihydrofluoride	25 g	Keep at RT
350-34923		100 g	

[References]

1) Hirao Ichiro., Kurumizaka Hitoshi., [Principles and protocols of nucleic acid experiments that can be selected according to purpose] Mokutekibetsu de eraberu kakusanjikken no genri to purotokoru (in Japanese). Yodosha Company, Ltd. (2011).

Purification Products

Liquid chromatography-based nucleic acid separation is widely used for oligonucleotide purification and analysis. Reversed-phase chromatography is based on the principle of separation using differences in hydrophobicity and uses a support of silica gel C18. Anion-exchange chromatography is based on the principle of separation using the polyanionic nature of nucleic acids and uses a support of an anion-exchange resin (rigid polymer, etc.) that is positively charged to capture anions.¹⁾

Solid-Phase Extraction column (SPE column)

Solid-phase extraction is a separation and purification technique to extract and purify the target material using a small-sized column packed with a silica gel or polymer gel support. During oligonucleotide pretreatment, the synthesized crude material is passed through a simple reversed-phase resin column (solid-phase extraction column) for contaminant removal and detritylation. This technique is suitable for pretreatment of small-volume samples.

Our Presep® DNA/RNA is a solid-phase extraction column suitable for oligonucleotide pretreatment. Type A uses a silica gel support and achieves high-efficiency and high-performance purification. It can be used as a sample pretreatment tool for HPLC or LC/MS analysis.

Features

- Achieves high sample load
- Can load a sample volume 3 to 5 times larger than that of commercial pretreatment columns
- Excellent deprotection efficiency
- High purification capacity
- High recovery



Fig 7. Presep® DNA/RNA Type A

Code No.	Product Name	Pkg. Size	Storage Condition
290-36691	Presep® DNA/RNA Type A (85 mg/1 mL)	20 pieces	Keep at RT
296-36693	Synthetic scale : 0.2-0.5 μ mol	50 pieces	
290-36711	Presep® DNA/RNA Type A (255 mg/3 mL)	20 pieces	Keep at RT
296-36713	Synthetic scale : 1-1.5 μ mol	50 pieces	
292-36891	Presep® DNA/RNA Type A (1.0 g/15 mL) Synthetic scale : 4-6 μ mol	10 pieces	Keep at RT
292-36911	Presep® DNA/RNA Type A (1.7 g/25 mL) Synthetic scale : 6-10 μ mol	10 pieces	Keep at RT
299-36921	Presep® DNA/RNA Type A (5.1 g/70 mL) Synthetic scale : 20-30 μ mol	10 pieces	Keep at RT

HPLC column (ODS column)

Wakopak® Ultra Series C18 is a highly durable ODS column using high-purity spherical silica gel.

Code No.	Product Name	Pkg. Size	Storage Condition
235-02651	Wakopak® Ultra C18-5 Φ 4.6 mm $\times$ 150 mm (W)	1 piece	Keep at RT

Eluents

A mixture of an organic solvent (acetonitrile, methanol, etc.) and a buffer (triethylamine-acetic acid, etc.) is used as an eluent for reversed-phase chromatography. As the buffer for nucleic acid separation, triethylamine-acetic acid, which is easily sublimated by freeze-drying, is generally used.¹⁾

Code No.	Product Name	Pkg. Size	Storage Condition
019-08631	Acetonitrile	1 L	Keep at RT
015-08633		3 L	
202-13131	2 mol/L Triethylamine Acetate Solution pH 7.0 【 TEAA solution 】	200 mL	Keep at RT
085-06991	1,1,1,3,3,3-Hexafluoro-2-propanol	100 mL	Keep at RT
087-06995		500 mL	

The products listed below are buffer products for nucleic acid synthesis manufactured and distributed by Nippon Gene Co., Ltd. These products can be used as dissolution solvents for salts or nucleic acids to be added to nucleic acid solutions for ethanol precipitation. Since we provide a custom buffer manufacturing service, we can manufacture custom-made buffers according to your request.

Code No.	Product Name	Pkg. Size	Storage Condition
316-90081	3 M Sodium Acetate (pH 5.2)	100 mL	Keep at RT
314-90021	TE (pH8.0) [10 mmol/L Tris-HCl (pH 8.0)1 mmol/L, EDTA (pH 8.0)]	100 mL	Keep at RT
310-90023		100 mL×6	
316-90025		500 mL	
316-90101	Distilled Water, Deionized	100 mL	Keep at RT
312-90103		100 mL×6	
318-90105		500 mL	

[References]

1) Hirao Ichiro., Kurumizaka Hitoshi., [Principles and protocols of nucleic acid experiments that can be selected according to purpose] Mokutekibetsu de eraberu kakusanjikken no genri to purotokoru (in Japanese). Yodosha Company, Ltd. (2011).

Drying traps

We offer zeolite packed in non-woven fabric pouches. By wrapping zeolite in non-woven fabric, crushed zeolite powder is prevented from being mixed into the solvent. After use, zeolite can be removed from the bottle with the non-woven fabric pouch, facilitating aftertreatment. Use these products for dehydration of amidite reagents or ancillary reagents.

- Shape: spherical
- 3 sizes: 3 g, 10 g, and 50 g
⇒ Selectable according to container size
- Highly airtight aluminum outer bag (degassed inside aluminum bag)
- Non-woven fabric material: composite fiber of polypropylene/polyethylene



Fig 8. Zeolite pack each capacity

Code No.	Product Name	Pkg. Size	Storage Condition
261-02271	Zeolite Packs [Zeolite, Synthetic, A-3, Beads, 1.40-2.36 mm (8 - 12mesh)] • Reference size of non-woven fabric 3 g : 60 mm×65 mm, 10 g : 120 mm×65 mm, 50 g : 120 mm×65 mm	3 g×20	Keep at RT
267-02273		10 g×20	
265-02274		50 g×10	

【Notes】

The moisture absorption capacity of synthetic zeolite is approximately 25% of its own weight. Add approximately 3 to 4 times the calculated amount of synthetic zeolite to your solvent.

Regular bottled products are also available. Please use them if non-woven fabric is not required.

Code No.	Product Name	Pkg. Size	Storage Condition
263-00575	Zeolite, Synthetic, A-3, Beads, 1.40 - 2.36 mm (8 - 12mesh)	500 g	Keep at RT
133-08645	Molecular Sieves 3A 1/8	500 g	Keep at RT
134-06095	Molecular Sieves 3A 1/16	500 g	Keep at RT