FUJIFILM
Value from Innovation

Wako

Presep® DNA/RNA Guide Book

Contents

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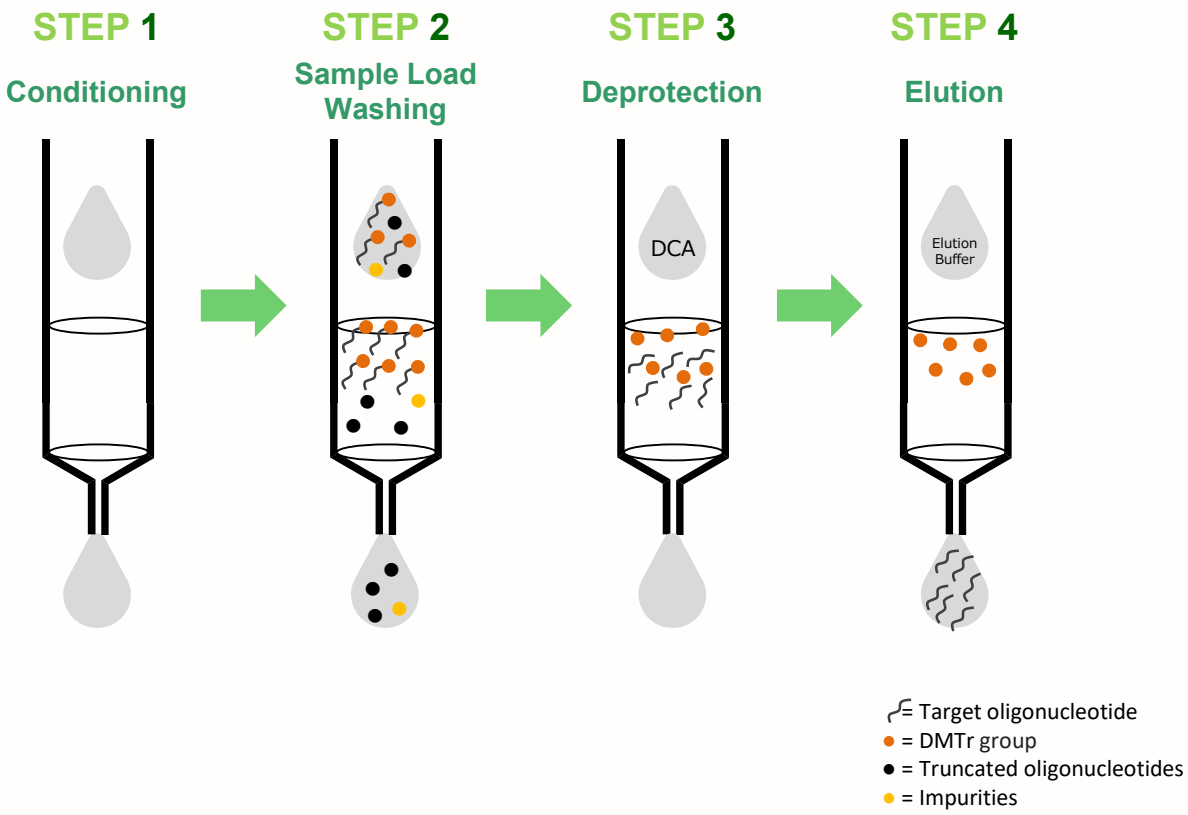
Nucleic Acid Purification by Solid-Phase Extraction

Solid-phase extraction (SPE) is a pretreatment technique based on the principles of liquid chromatography. It involves passing a sample solution through a column packed with a selective separation medium to extract only the target component. Due to its ability to purify samples rapidly and conveniently, SPE is widely used in the pretreatment of samples in various analytical techniques, including HPLC, GC, and LC/MS.

In oligonucleotide synthesis, SPE is employed as a straightforward purification technique, contributing to efficient purification and enhancement of analytical precision.

During the synthesis of oligonucleotides, the 4,4′-Dimethoxytrityl (DMTr) group at the 5′ end can be removed or retained, which dictates the choice of purification process. When the DMTr group is removed, the oligonucleotide is cleaved from the solid support, deprotected, and then separated from impurities (such as unreacted nucleic acid fragments) and purified using HPLC. On the other hand, retaining the DMTr group allows for efficient removal of impurities by SPE. For example, after cleaving from the solid support, passing the solution through a simple reverse-phase column allows only the nucleic acid fragments with a DMTr group to be retained on the resin, thereby eliminating unreacted nucleic acid fragments. Subsequently, the DMTr group is removed by treatment with an acid such as trichloroacetic acid, trifluoroacetic acid, or dichloroacetic acid, followed by elution of the target nucleic acid. Thus, when the DMTr group is retained during synthesis, SPE has become the standard method for simplified oligonucleotide purification due to its efficiency in removing impurities.

Nucleic Acid SPE Procedure



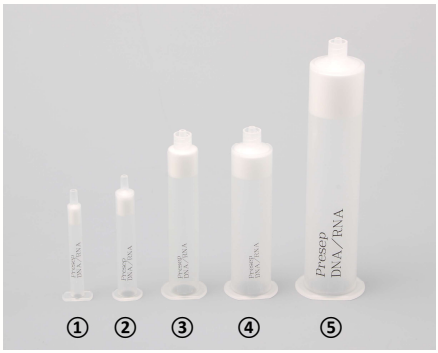
Presep® DNA/RNA Type A

Presep® DNA/RNA are SPE columns designed for the separation and purification of nucleic acids. The Type A columns are mini-columns filled with a silica-based separation medium in a syringe-style format, intended for use in reverse-phase SPE.

Features

- Achieves a high loading capacity, allowing for 3-5 times greater sample load compared to other commercially available pretreatment columns.
- Offers superior deprotection efficiency.
- Enables purification with high purity and high recovery.

Appearance



Column Capacity

No.	Particle Support Weight / Syringe Volume	Synthesis Scale Compatibility
①	85 mg / 1 mL	0.2-0.5 μmol
②	255 mg / 3 mL	1-1.5 μmol
③	1.0 g / 15 mL	4-6 μmol
④	1.7 g / 25 mL	6-10 μmol
⑤	5.1 g / 70 mL	20-30 μmol

Product List

Code No.	Product Name	Grade	Package Size
290-36691	Presep® DNA/RNA Type A (85 mg/1 mL)	for Nucleic Acid Synthesis	20 pcs
296-36693			50 pcs
290-36711	Presep® DNA/RNA Type A (255 mg/3 mL)	for Nucleic Acid Synthesis	20 pcs
296-36713			50 pcs
292-36891	Presep® DNA/RNA Type A (1.0 g/15 mL)	for Nucleic Acid Synthesis	10 pcs
292-36911	Presep® DNA/RNA Type A (1.7 g/25 mL)	for Nucleic Acid Synthesis	10 pcs
299-36921	Presep® DNA/RNA Type A (5.1 g/70 mL)	for Nucleic Acid Synthesis	10 pcs

How to Use SPE Columns

Presep® DNA/RNA should be used with a suction device, whereby reduced pressure is applied to the outlet side of the column. The use of gravity for elution is not appropriate. For processing multiple samples, use of a suction manifold is recommended.

SPE Conditions

■Condition 1

Presep® DNA/RNA Type A **(85 mg / 1 mL)**

Specification	
Particle Support	Silica gel
Cartridge Type	Syringe
Syringe Size	5.5 φ × 57 mm
Synthesis Scale Compatibility	0.2-0.5 μmol

Steps	Materials	Amount Used
Conditioning	Acetonitrile	1 mL
	100 mg/mL NaCl aq.	1 mL × 2
Sample Load	Sample solution 0.5 mL + 100 mg/mL NaCl aq. 0.5 mL	1mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=5/95(v/v)	1 mL × 2
Deprotection	2% DCA*1 aq. (DCA/Water=2/98(v/v))	1 mL
Washing 2	RNase Free Water	1 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*2	1 mL

■Condition 2

Presep® DNA/RNA Type A **(255 mg / 3 mL)**

Specification	
Particle Support	Silica gel
Cartridge Type	Syringe
Syringe Size	9.0 φ × 63 mm
Synthesis Scale Compatibility	1-1.5 μmol

Steps	Materials	Amount Used
Conditioning	Acetonitrile	3 mL
	100 mg/mL NaCl aq.	3 mL × 2
Sample Load	Sample solution 1 mL + 100 mg/mL NaCl aq. 1 mL	2 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=5/95(v/v)	3 mL × 2
Deprotection	2% DCA*1 aq. (DCA/Water=2/98(v/v))	3 mL
Washing 2	RNase Free Water	3 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*2	3 mL

■Condition 3

Presep® DNA/RNA Type A **(1.0 g / 15 mL)**

Specification	
Particle Support	Silica gel
Cartridge Type	Syringe
Syringe Size	15 φ × 87 mm
Synthesis Scale Compatibility	4-6 μmol

Steps	Materials	Amount Used
Conditioning	Acetonitrile	12 mL
	100 mg/mL NaCl aq.	12 mL × 2
Sample Load	Sample solution 5 mL + 100 mg/mL NaCl aq. 5 mL	10 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=5/95(v/v)	12 mL × 2
Deprotection	2% DCA*1 aq. (DCA/Water=2/98(v/v))	12 mL
Washing 2	RNase Free Water	12 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*2	12 mL

■Condition 4

Presep® DNA/RNA Type A **(1.7 g / 25 mL)**

Specification	
Particle Support	Silica gel
Cartridge Type	Syringe
Syringe Size	21 φ × 85 mm
Synthesis Scale Compatibility	6-10 μmol

Steps	Materials	Amount Used
Conditioning	Acetonitrile	20 mL
	100 mg/mL NaCl aq.	20 mL × 2
Sample Load	Sample solution 10 mL + 100 mg/mL NaCl aq. 10 mL	20 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=5/95(v/v)	20 mL × 2
Deprotection	2% DCA*1 aq. (DCA/Water=2/98(v/v))	20 mL
Washing 2	RNase Free Water	20 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*2	20 mL

■Condition 5

Presep® DNA/RNA Type A **(5.1 mg / 70 mL)**

Specification	
Particle Support	Silica gel
Cartridge Type	Syringe
Syringe Size	27 φ × 134 mm
Synthesis Scale Compatibility	20-30 μmol

Steps	Materials	Amount Used
Conditioning	Acetonitrile	60 mL
	100 mg/mL NaCl aq.	60 mL × 2
Sample Load	Sample solution 20 mL + 100 mg/mL NaCl aq. 20 mL	40 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=5/95(v/v)	60 mL × 2
Deprotection	2% DCA*1 aq. (DCA/Water=2/98(v/v))	60 mL
Washing 2	RNase Free Water	60 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*2	60 mL

*1 DCA = Dichloroacetic Acid
*2 Add 50 μL of 28% ammonium hydroxide per 10 mL of 50% acetonitrile in water.

Comparison of Separation Capabilities

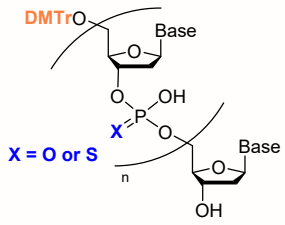
Experiments were conducted to compare Presep® DNA/RNA Type A with SPE columns from other companies.

1. Oligonucleotide Recovery

Sample of Cartridge Column

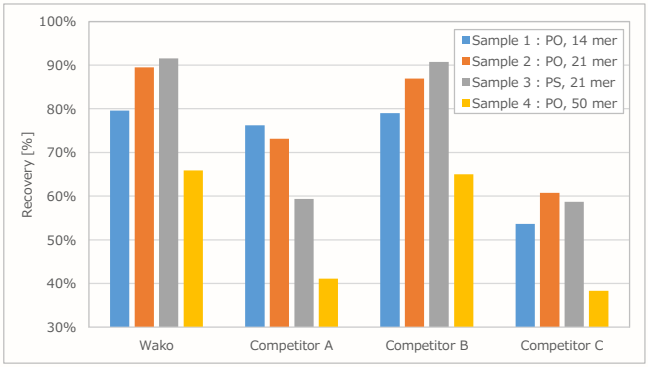
	Wako (Presep® DNA/RNA Type A)	Competitor A	Competitor B	Competitor C
Particle Support	Silica gel	Silica gel	Polymer	Polymer

Sample of Oligonucleotide



Sample Name	Length	Linkage Structure
Sample 1	14 mer	PO
Sample 2	21 mer	PO
Sample 3	21 mer	PS
Sample 4	50 mer	PO

Cartridge Column *vs* Recovery of oligonucleotide [%]



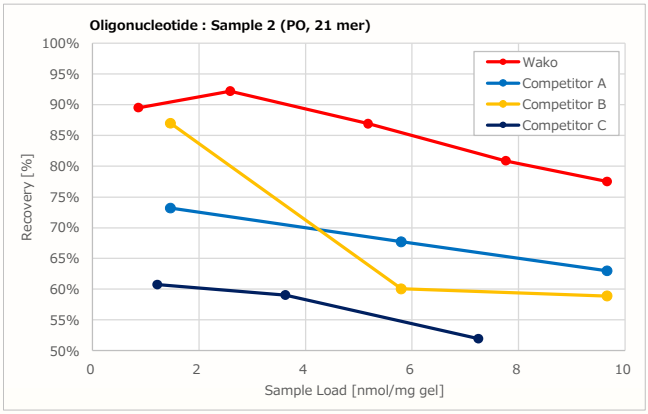
Presep® DNA/RNA Type A achieved high recovery.

Wako ≒ B > A > C

- Oligonucleotide samples were purified using the recommended protocols from each company.
- The concentration of oligonucleotides was measured with a spectrophotometer.
- Recovery was calculated by dividing the concentration of oligonucleotides in the eluate by the pre-loading concentration of oligonucleotides.

2. Oligonucleotide Loading Capacity

Sample Load *vs* Recovery of oligonucleotide [%]



Presep® DNA/RNA Type A has a high loading capacity.

When the loading ratio of oligonucleotides to resin was increased to 9.7, the recovery for products from other companies (A, B, C) dropped below 65%, whereas the FUJIFILM Wako's product maintained a recovery exceeding 75%.

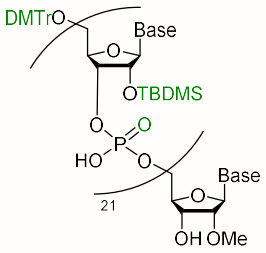
Sample Information of Oligonucleotide

No.	Sample Name	Scale	Length	Linkage Structure	Ribose modification	Page
1	PO RNA Oligonucleotide (DMT-ON)	0.2 μmol	22 mer	PO	—	6
2	PO RNA Oligonucleotide (DMT-ON)	1 μmol	22 mer	PO	—	7
3	PS RNA Oligonucleotide (DMT-ON)	0.2 μmol	22 mer	PS	—	8
4	PS RNA Oligonucleotide (DMT-ON)	1 μmol	22 mer	PS	—	9
5	PO/PS DNA Oligonucleotide (DMT-ON)	0.5 μmol	20 mer	PO/PS mix	—	10
6	PO/PS DNA Oligonucleotide (DMT-ON)	5 μmol	20 mer	PO/PS mix	—	11
7	PO/PS DNA Oligonucleotide (DMT-ON)	10 μmol	20 mer	PO/PS mix	—	12
8	LNA/2'-OMe Mixmer RNA Oligonucleotide (DMT-ON)	0.2 μmol	18 mer	PS	LNA, 2'-OMe	13
9	LNA/2'-OMe Mixmer RNA Oligonucleotide (DMT-ON)	1 μmol	18 mer	PS	LNA, 2'-OMe	14
10	LNA/2'-OMe Mixmer RNA Oligonucleotide (DMT-ON)	20 μmol	18 mer	PS	LNA, 2'-OMe	15
11	LNA/DNA Gapmer Oligonucleotide (DMT-ON)	0.5 μmol	16 mer	PS	LNA	16
12	LNA/DNA Gapmer Oligonucleotide (DMT-ON)	5 μmol	16 mer	PS	LNA	17
13	LNA/DNA Gapmer Oligonucleotide (DMT-ON)	10 μmol	16 mer	PS	LNA	18

No. 1　0.2 μmol scale PO RNA Oligonucleotide (DMT-ON)

Sample

Sample of oligonucleotide：DMT-ON 2'-TBDMS RNA 22 mer, All PO
Synthesis Scale：0.2 μmol



Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 10 μmol scale.
(2) Add 5 mL of AMA* solution to (1) and heat at 65°C for 1 hour to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 10 mL.
(3) Take 0.2 mL (0.2 μmol scale) from (2), and concentrate to dryness by centrifugal evaporation.
(4) Dissolve the dried DMT-ON form in 20 μL of DMSO. Then add 10 μL of TEA.
(5) Add 10 μL of TEA·3HF and heat at 65°C for 2.5 hours to desilylate the 2′ position. Cool the reaction mixture afterward.
(6) Quench with 0.25 mL of 10% NH_4OH, then add an equal volume (0.25 mL) of 100 mg/mL NaCl(aq).
(7) Load the DMT-ON form (**total volume 0.54 mL**) onto an SPE column for purification.

*AMA : Ammonium hydroxide/Methylamine (Ammonium hydroxide = 28% NH_3 in H_2O)

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (85 mg / 1 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	1 mL
	100 mg/mL NaCl aq.	1 mL × 2
Sample Load	Sample solution 0.54 mL	0.54 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=5/95(v/v)	1 mL × 2
Deprotection	2% DCA aq. (DCA/Water=2/98(v/v))	1 mL
Washing 2	RNase Free Water	1 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*	1 mL

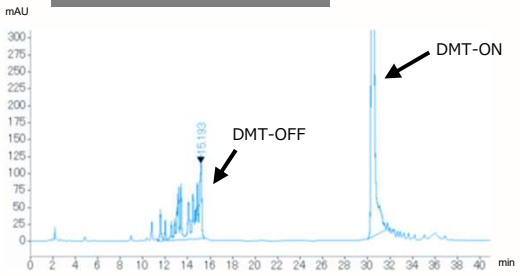
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 50% acetonitrile in water.

HPLC analysis

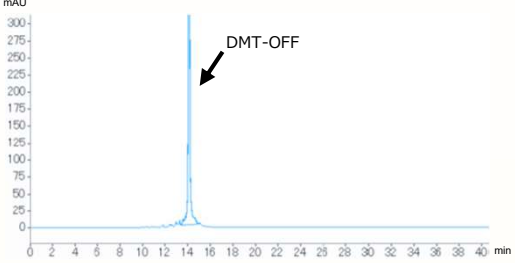
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification



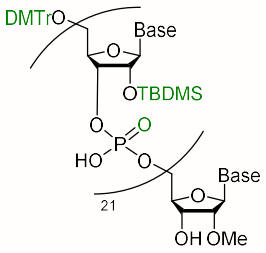
b) After purification



No. 2　1 μmol scale PO RNA Oligonucleotide (DMT-ON)

Sample

Sample of oligonucleotide：DMT-ON 2'-TBDMS RNA 22 mer, All PO
Synthesis Scale：1 μmol



Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 10 μmol scale.
(2) Add 5 mL of AMA* solution to (1) and heat at 65°C for 1 hour to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 10 mL.
(3) Take 1 mL (1 μmol scale) from (2), and concentrate to dryness by centrifugal evaporation.
(4) Dissolve the dried DMT-ON form in 100 μL of DMSO. Then add 50 μL of TEA.
(5) Add 50 μL of TEA·3HF and heat at 65°C for 2.5 hours to desilylate the 2′ position. Cool the reaction mixture afterward.
(6) Quench with 1.4 mL of 10% NH_4OH, then add an equal volume (1.4 mL) of 100 mg/mL NaCl(aq).
(7) Load the DMT-ON form (**total volume 3 mL**) onto an SPE column for purification.

*AMA : Ammonium hydroxide/Methylamine (Ammonium hydroxide = 28% NH_3 in H_2O)

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (255 mg / 3 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	3 mL
	100 mg/mL NaCl aq.	3 mL × 2
Sample Load	Sample solution 3 mL	3 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=5/95(v/v)	3 mL × 2
Deprotection	2% DCA aq. (DCA/Water=2/98(v/v))	3 mL
Washing 2	RNase Free Water	3 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*	3 mL

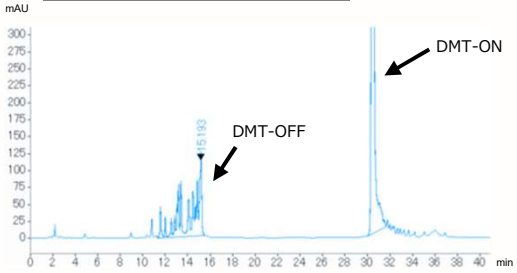
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 50% acetonitrile in water.

HPLC analysis

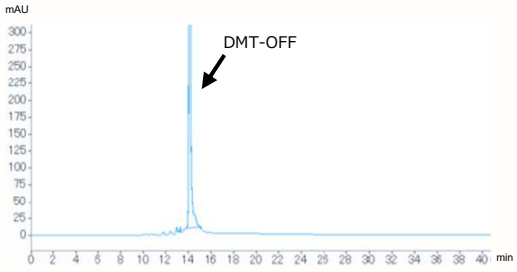
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification



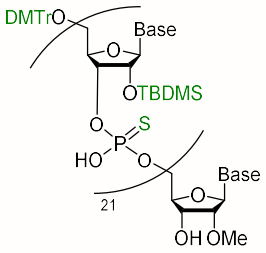
b) After purification



No. 3　0.2 μmol scale PS RNA Oligonucleotide (DMT-ON)

Sample

Sample of oligonucleotide：DMT-ON 2'-TBDMS RNA 22 mer, All PS
Synthesis Scale：0.2 μmol



Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 10 μmol scale.
(2) Add 5 mL of AMA* solution to (1) and heat at 65°C for 1 hour to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 10 mL.
(3) Take 0.2 mL (0.2 μmol scale) from (2), and concentrate to dryness by centrifugal evaporation.
(4) Dissolve the dried DMT-ON form in 20 μL of DMSO. Then add 10 μL of TEA.
(5) Add 10 μL of TEA·3HF and heat at 65°C for 2.5 hours to desilylate the 2′ position. Cool the reaction mixture afterward.
(6) Quench with 0.25 mL of 10% NH_4OH, then add an equal volume (0.25 mL) of 100 mg/mL NaCl(aq).
(7) Load the DMT-ON form (**total volume 0.54 mL**) onto an SPE column for purification.

*AMA : Ammonium hydroxide/Methylamine (Ammonium hydroxide = 28% NH_3 in H_2O)

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (85 mg / 1 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	1 mL
	100 mg/mL NaCl aq.	1 mL × 2
Sample Load	Sample solution 0.54 mL	0.54 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	1 mL × 2
Deprotection	2% DCA aq. (DCA/Water=2/98(v/v))	1 mL
Washing 2	RNase Free Water	1 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*	1 mL

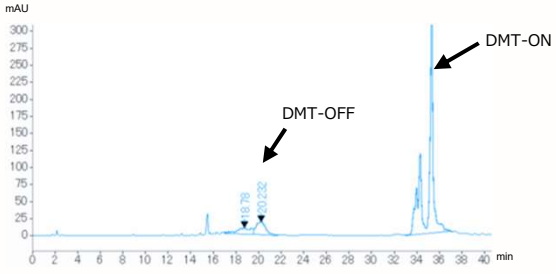
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 50% acetonitrile in water.

HPLC analysis

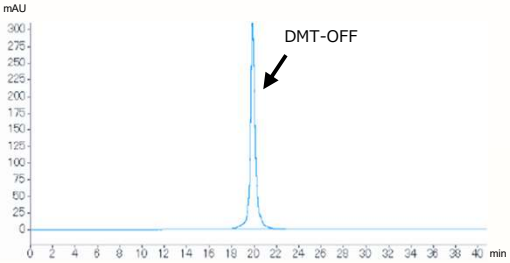
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification



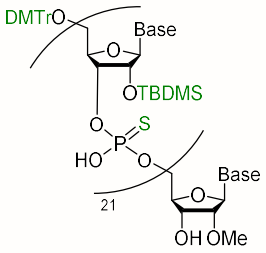
b) After purification



No. 4　1 μmol scale PS RNA Oligonucleotide (DMT-ON)

Sample

Sample of oligonucleotide：DMT-ON 2'-TBDMS RNA 22 mer, All PS
Synthesis Scale：1 μmol



Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 10 μmol scale.
(2) Add 5 mL of AMA* solution to (1) and heat at 65°C for 1 hour to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 10 mL.
(3) Take 1 mL (1 μmol scale) from (2), and concentrate by centrifugal evaporation.
(4) Dissolve the dried DMT-ON form in 100 μL of DMSO. Then add 50 μL of TEA.
(5) Add 50 μL of TEA·3HF and heat at 65°C for 2.5 hours to desilylate the 2′ position. Cool the reaction mixture afterward.
(6) Quench with 1.4 mL of 10% NH_4OH, then add an equal volume (1.4 mL) of 100 mg/mL NaCl(aq).
(7) Load the DMT-ON form (**total volume 3 mL**) onto an SPE column for purification.

*AMA : Ammonium hydroxide/Methylamine (Ammonium hydroxide = 28% NH_3 in H_2O)

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (255 mg / 3 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	3 mL
	100 mg/mL NaCl aq.	3 mL × 2
Sample Load	Sample solution 3 mL	3 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	3 mL × 2
Deprotection	2% DCA aq. (DCA/Water=2/98(v/v))	3 mL
Washing 2	RNase Free Water	3 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=50/50(v/v)*	3 mL

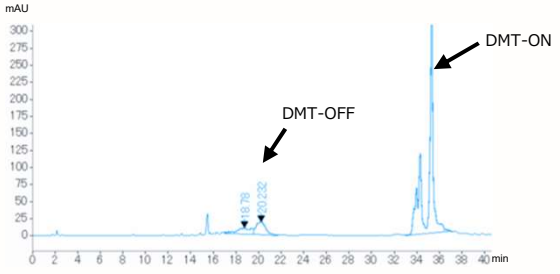
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 50% acetonitrile in water.

HPLC analysis

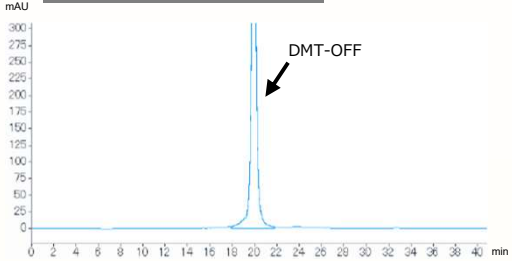
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification



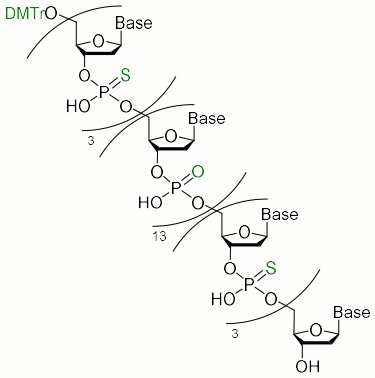
b) After purification



No. 5　0.5 μmol scale PO/PS DNA Oligonucleotide (DMT-ON)

Sample

Sample of oligonucleotide：DMT-ON PO/PS DNA 20 mer
Synthesis Scale：0.5 μmol



Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 20 μmol scale.
(2) Add 10 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 20 mL.
(3) Take 0.5 mL (0.5 μmol scale) from (2), then add an equal volume (0.5 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 1 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (85 mg / 1 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	1 mL
	100 mg/mL NaCl aq.	1 mL × 2
Sample Load	Sample solution 1 mL	1 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=8/92(v/v)	1 mL × 2
Deprotection	2% DCA aq. (DCA/Water=2/98(v/v))	1 mL
Washing 2	RNase Free Water	1 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	1 mL

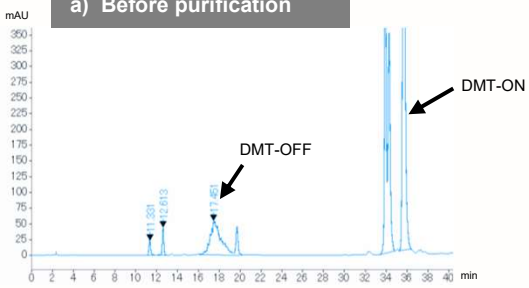
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

HPLC analysis

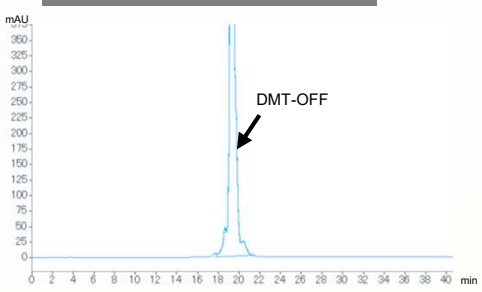
Condition

Column size : Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase : A) 100 mmol/L TEAA aq.
: B) Acetonitrile
Gradient : 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate : 1.0 mL/min
Temperature : 30℃
Injection vol. : 2 μL
Sample : a) Sample before purification
b) Sample after purification

a) Before purification

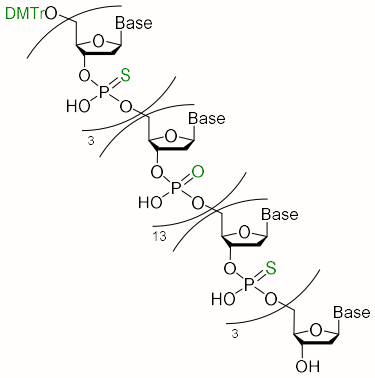


b) After purification



No. 6　5 μmol scale PO/PS DNA Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide：DMT-ON PO/PS DNA 20 mer
Synthesis Scale：5 μmol

Procedure

Sample Solution Preparation

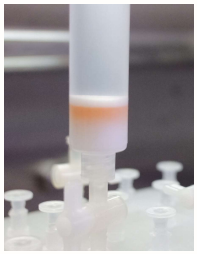
(1) Prepare crude oligonucleotide synthesized on a 20 μmol scale.
(2) Add 10 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 20 mL.
(3) Take 5 mL (5 μmol scale) from (2), then add an equal volume (5 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 10 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (1 g / 15 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	12 mL
	100 mg/mL NaCl aq.	12 mL × 2
Sample Load	Sample solution 10 mL	10 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=8/92(v/v)	12 mL × 2
Deprotection	2% DCA aq. (DCA/Water=2/98(v/v))	12 mL
Washing 2	RNase Free Water	12 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	12 mL

*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

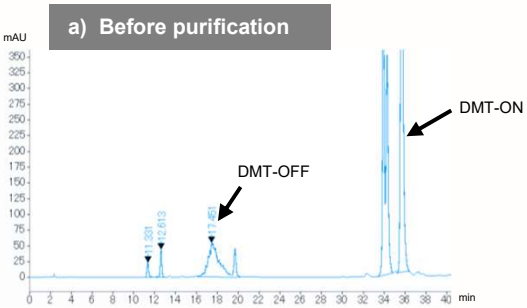


HPLC analysis

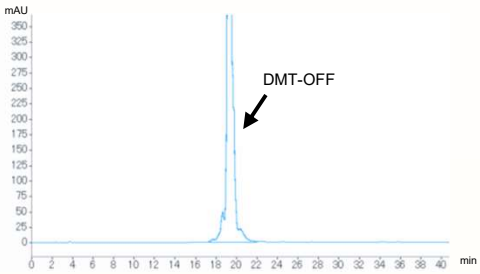
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification

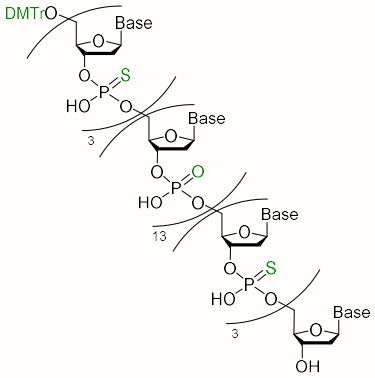


b) After purification



No. 7　10 μmol scale PO/PS DNA Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide：DMT-ON PO/PS DNA 20 mer
Synthesis Scale：10 μmol

Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 20 μmol scale.
(2) Add 10 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 20 mL.
(3) Take 10 mL (10 μmol scale) from (2), then add an equal volume (10 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 20 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (1.7 mg / 25 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	20 mL
	100 mg/mL NaCl aq.	20 mL × 2
Sample Load	Sample solution 20 mL	20 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=8/92(v/v)	20 mL × 2
Deprotection	2% DCA aq. (DCA/Water=2/98(v/v))	20 mL
Washing 2	RNase Free Water	20 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	20 mL

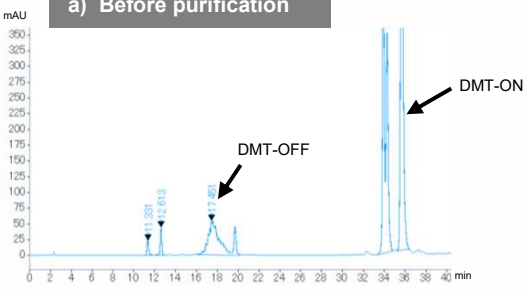
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

HPLC analysis

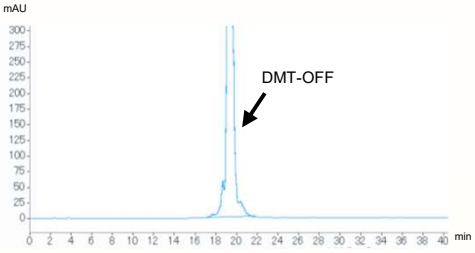
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification

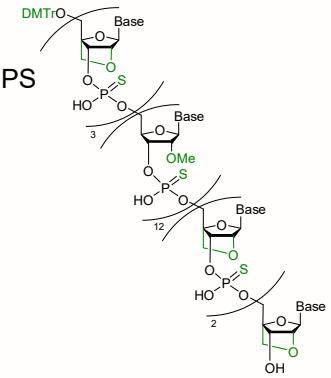


b) After purification



No. 8　0.2 μmol scale LNA/2'-OMe Mixmer RNA Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide：DMT-ON LNA/2'-OMe RNA Mixmer 18 mer, All PS
Synthesis Scale：0.2 μmol

Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 10 μmol scale.
(2) Add 5 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 10 mL.
(3) Take 0.2 mL (0.2 μmol scale) from (2), then add an equal volume (0.2 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 0.4 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (85 mg / 1 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	1 mL
	100 mg/mL NaCl aq.	1 mL × 2
Sample Load	Sample solution 0.4 mL	0.4 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	1 mL × 2
Deprotection	3% DCA aq. (DCA/Water=3/97(v/v)) (Wait 30 seconds for each 1 mL)	1 mL × 2
Washing 2	RNase Free Water	1 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	1 mL

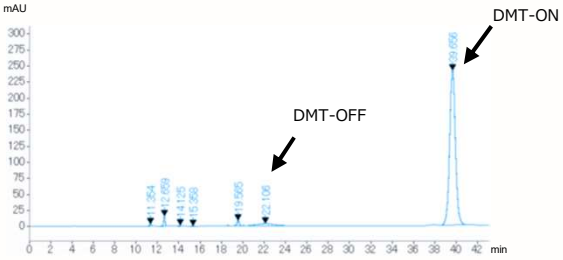
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

HPLC analysis

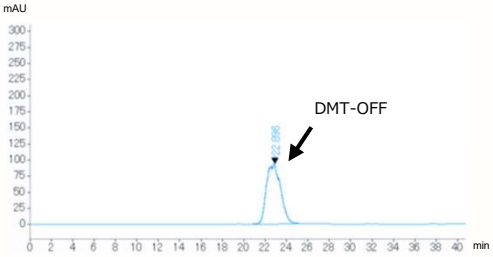
Condition

Column size : Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase : A) 100 mmol/L TEAA aq.
: B) Acetonitrile
Gradient : 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate : 1.0 mL/min
Temperature : 30℃
Injection vol. : 2 μL
Sample : a) Sample before purification
b) Sample after purification

a) Before purification

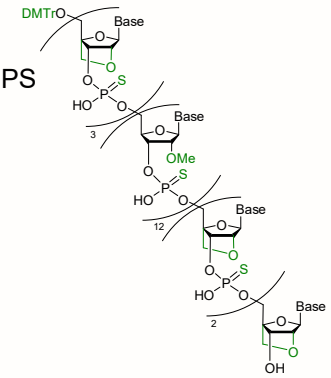


b) After purification



No. 9　1 μmol scale LNA/2'-OMe Mixmer RNA Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide：DMT-ON LNA/2'-OMe RNA Mixmer 18 mer, All PS
Synthesis Scale：1 μmol

Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 10 μmol scale.
(2) Add 5 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 10 mL.
(3) Take 1 mL (1 μmol scale) from (2), then add an equal volume (1 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 2 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (255 mg / 3 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	3 mL
	100 mg/mL NaCl aq.	3 mL × 2
Sample Load	Sample solution 2 mL	2 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	3 mL × 2
Deprotection	3% DCA aq. (DCA/Water=3/97(v/v)) (Wait 30 seconds for each 3 mL)	3 mL × 2
Washing 2	RNase Free Water	3 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	3 mL

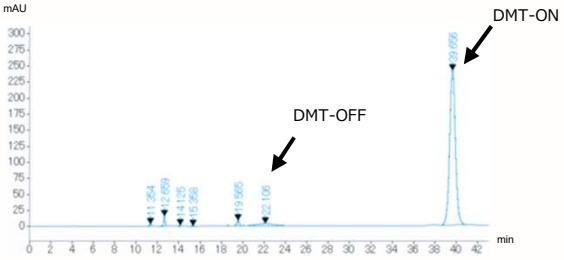
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

HPLC analysis

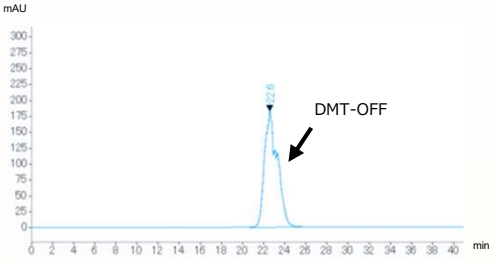
Condition

Column size : Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase : A) 100 mmol/L TEAA aq.
: B) Acetonitrile
Gradient : 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate : 1.0 mL/min
Temperature : 30℃
Injection vol. : 2 μL
Sample : a) Sample before purification
b) Sample after purification

a) Before purification

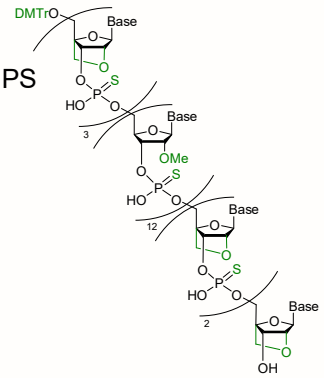


b) After purification



No. 10　20 μmol scale LNA/2'-OMe Mixmer RNA Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide :　DMT-ON LNA/2'-OMe RNA mixmer 18 mer, All PS
Synthesis Scale : 20 μmol

Procedure

Sample Solution Preparation

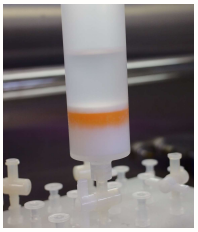
(1) Prepare crude oligonucleotide synthesized on a 20 μmol scale.
(2) Add 10 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 10 mL.
(3) Take 20 mL (20 μmol scale) from (2), then add an equal volume (1 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 40 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (5.1 mg / 70 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	60 mL
	100 mg/mL NaCl aq.	60 mL × 2
Sample Load	Sample solution 40 mL	40 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	60 mL × 2
Deprotection	3% DCA aq. (DCA/Water=3/97(v/v)) (Wait 30 seconds for each 60 mL)	60 mL × 3
Washing 2	RNase Free Water	60 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	60 mL

*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

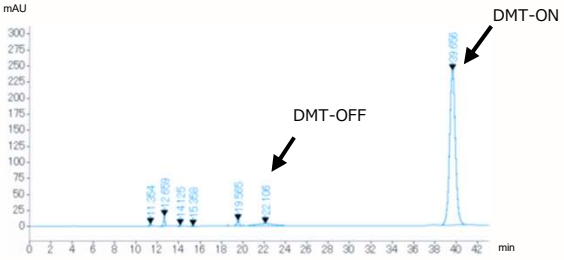


HPLC analysis

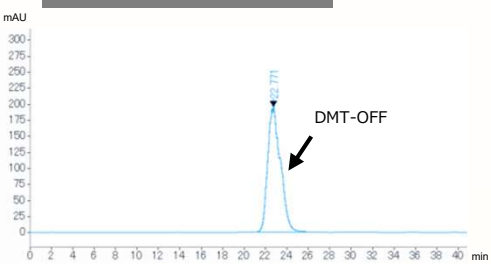
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification

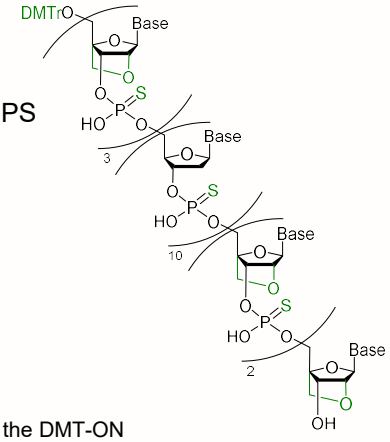


b) After purification



No. 11　0.5 μmol scale LNA/DNA Gapmer Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide：DMT-ON LNA/DNA Gapmer 16 mer, All PS
Synthesis Scale：0.5 μmol

Procedure

Sample Solution Preparation

(1) Prepare crude oligonucleotide synthesized on a 20 μmol scale.
(2) Add 10 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 20 mL.
(3) Take 0.5 mL (0.5 μmol scale) from (2), then add an equal volume (0.5 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 1 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (85 mg / 1 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	1 mL
	100 mg/mL NaCl aq.	1 mL × 2
Sample Load	Sample solution 1 mL	1 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	1 mL × 2
Deprotection	3% DCA aq. (DCA/Water=3/97(v/v)) (Wait 30 seconds for each 1 mL)	1 mL × 2
Washing 2	RNase Free Water	1 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	1 mL

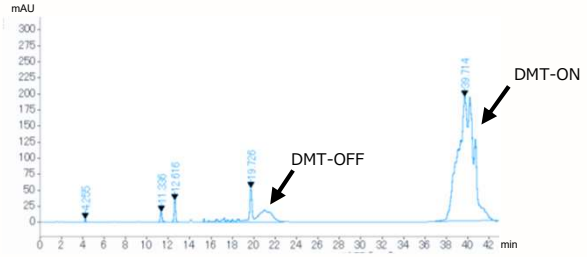
*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

HPLC analysis

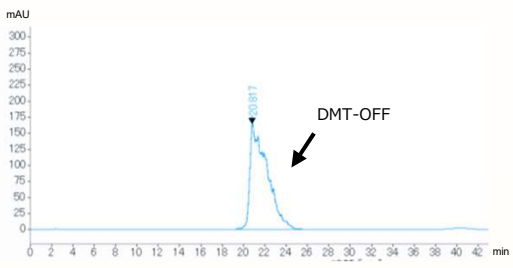
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification

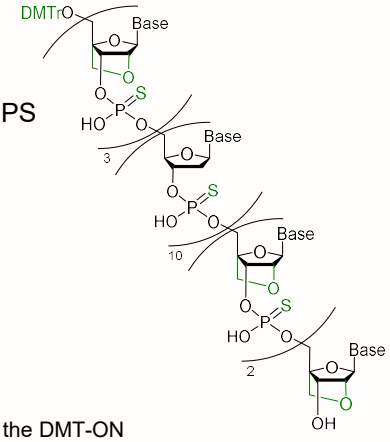


b) After purification



No. 12　5 μmol scale LNA/DNA Gapmer Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide：DMT-ON LNA/DNA Gapmer 16 mer, All PS
Synthesis Scale：5 μmol

Procedure

Sample Solution Preparation

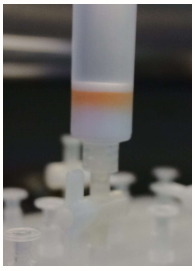
(1) Prepare crude oligonucleotide synthesized on a 20 μmol scale.
(2) Add 10 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 20 mL.
(3) Take 5 mL (5 μmol scale) from (2), then add an equal volume (5 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 10 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (1 g / 15 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	12 mL
	100 mg/mL NaCl aq.	12 mL × 2
Sample Load	Sample solution 10 mL	10 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	12 mL × 2
Deprotection	3% DCA aq. (DCA/Water=3/97)(v/v) (Wait 30 seconds for each 1 mL)	12 mL × 2
Washing 2	RNase Free Water	12 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	12 mL

*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

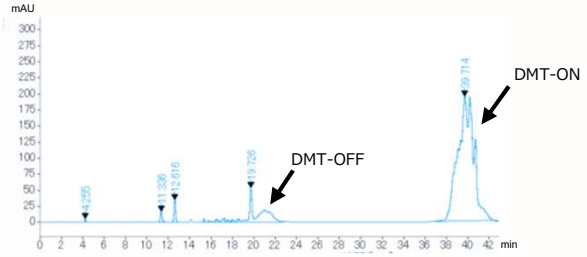


HPLC analysis

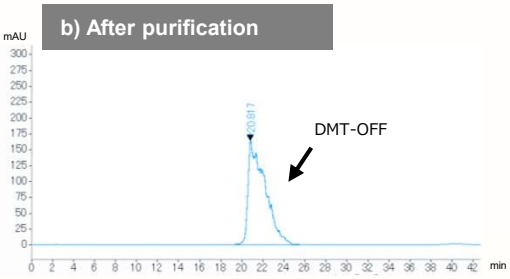
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification

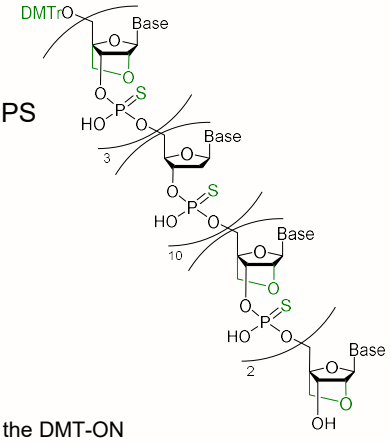


b) After purification



No. 13　10 μmol scale LNA/DNA Gapmer Oligonucleotide (DMT-ON)

Sample



Sample of oligonucleotide：DMT-ON LNA/DNA Gapmer 16 mer, All PS
Synthesis Scale：10 μmol

Procedure

Sample Solution Preparation

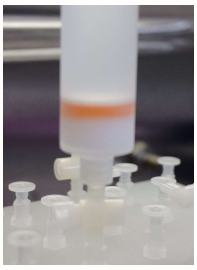
(1) Prepare crude oligonucleotide synthesized on a 20 μmol scale.
(2) Add 10 mL of 28% NH_4OH solution to (1) and heat at 55°C for 6 hours to cleave the DMT-ON form from the solid support. Then, dilute with ultra-pure water (RNase-free water) to double the volume to 20 mL.
(3) Take 10 mL (10 μmol scale) from (2), then add an equal volume (10 mL) of 100 mg/mL NaCl(aq).
(4) Load the DMT-ON form (**total volume 20 mL**) onto an SPE column for purification.

SPE Procedure

SPE cartridge : Presep® DNA/RNA Type A (1.7 mg / 25 mL)

Steps	Materials	Amount Used
Conditioning	Acetonitrile	20 mL
	100 mg/mL NaCl aq.	20 mL × 2
Sample Load	Sample solution 20 mL	20 mL
Washing 1	Acetonitrile/100 mg/mL NaCl aq.=10/90(v/v)	20 mL × 2
Deprotection	3% DCA aq. (DCA/Water=3/97(v/v)) (Wait 30 seconds for each 20 mL)	20 mL × 2
Washing 2	RNase Free Water	20 mL × 2
Elution	0.5% NH_4OH in Acetonitrile/Water=15/85(v/v)*	20 mL

*Add 50 μL of 28% ammonium hydroxide per 10 mL of 15% acetonitrile in water.

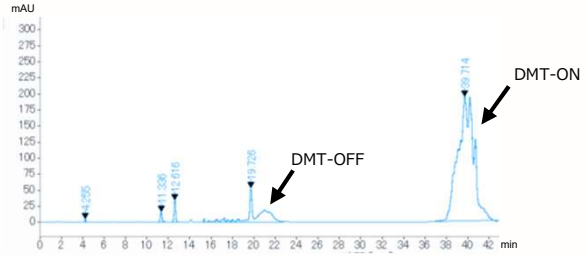


HPLC analysis

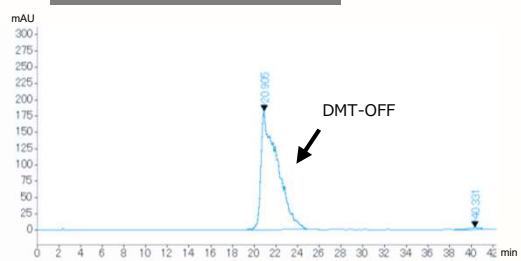
Condition

Column size　: Wakopak Wakosil® 5C18 4.6 φ x 150 mm
Mobile phase　: A) 100 mmol/L TEAA aq.
　　: B) Acetonitrile
Gradient　: 0-45 min B=5-40%, 45-50 min B=100%, 50-60 min B=5%
Flow rate　: 1.0 mL/min
Temperature　: 30℃
Injection vol.　: 2 μL
Sample　: a) Sample before purification
　　b) Sample after purification

a) Before purification



b) After purification



Related product

- **Cleavage Reagents**

Code No.	Product Name	Grade CAS RN®	Package Size
017-03176	25% Ammonia Solution	Wako 1st Grade 1336-21-6	500mL
132-01851	40% Methylamine Solution	– 74-89-5	100mL

Reagents for Nucleic Acid Synthesis

FUJIFILM Wako 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.

For details, feel free to check out our homepage.

https://labchem-wako.fujifilm.com/us/category/synthesis/nucleicacid/index.html

FUJIFILM Wako Laboratory Chemicals site
https://labchem-wako.fujifilm.com

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