Products for DNA Research

User Guide to Glen-Pak™ Purification

GLEN RESEARCH

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Contents

Principles of Glen-Pak™ DNA/RNA Purification

As with Poly-Pak™ cartridges, Glen-Pak™ cartridges utilize the 5' DMT retained on an oligonucleotide to specifically bind full-length sequences to the support. During the purification process, the failure sequences are eliminated and the DMT is subsequently removed, allowing for elution of purified product.

There are many benefits to the Glen-Pak purification system over conventional Solid Phase Extraction (SPE) systems.

Versatility

Purify oligonucleotides directly without any pre-purification drying steps from:

- Ammonium Hydroxide
- AMA (Ammonium hydroxide/40% Aqueous Methylamine 1:1)
- Tert-Butylamine/water 1:3 (v/v) (1)
- 50 mM Potassium Carbonate in Methanol (2)
- 0.4M NaOH in Methanol/Water 4:1 (v/v) (3)

using cartridges fitted for a handheld syringes, vacuum manifolds or high-throughput 96-well plates.

(1) Dilute t-Butylamine/water solution with 100 mg/mL Sodium Chloride 1:7 (v/v) prior to loading.

(2) Dilute 50 mM Potassium Carbonate in Methanol with 100 mg/mL Sodium Chloride 1:4 (v/v) prior to loading

(3) Dilute 1.25mL final deprotection volume to 10mL with 100 mg/mL Sodium Chloride prior to loading.

Capacity

- Ability to purify longer oligos - we have successfully purified oligos up to 150 bases in length using the DNA cartridge and over 60 bases on the RNA cartridge
- Ability to efficiently purify short and long oligos from 40 nmole up to 1.0 µmole scale using the same protocol

When using a vacuum manifold, adjust the pressure to ~7mm Hg using the vacuum control valve (if no control valve is available on your manifold, or you are using a disposable syringe version of the Glen-Pak, target a flow rate of about 1-2 drops per second).

Scale Suggestions for Glen-Pak DNA Cartridges

Glen-Pak DNA Product	Catalog Number	Synthesis Scale Compatibility
Glen-Pak DNA 50mg Purification Cartridge	60-5000-96	10 nmole – 200 nmole
Glen-Pak DNA Purification Cartridge	60-5100-XX and 60-5200-XX	10 nmole – 1.0 µmole
Glen-Pak DNA Cartridge 3G	60-5300-01	5 µmole – 20 µmole
Glen-Pak DNA 30 mg 96-Well Plate	60-5400-01	10 nmole – 50 nmole

Purification of DNA Oligonucleotides (DMT-ON)

Materials

Materials	Amount Used
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type, if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	1mL
Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
2% Trifluoroacetic Acid (TFA)/Water (60-4040-57)	2mL
Deionized Water	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL

* Add 50µL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

Sample Preparation

1. Following DNA synthesis, deprotect the DMT-ON oligonucleotide in 1mL of ammonium hydroxide or ammonium hydroxide/methylamine (AMA) as normal. There is no need to lyophilize the deprotection solution. *For this procedure to be successful, the synthesis must have been carried out DMT-ON. The base labile protecting groups must have been removed with either AMA or ammonium hydroxide (1.0mL).*
2. Add 1mL of 100 mg/mL Sodium Chloride solution to the deprotected DMT-ON oligonucleotide for a final volume of 2mL. *The final salt concentration of the sample must be around 50 mg/mL for the loading of the oligo on the cartridge. Larger volumes may be loaded, but this is our suggested volume for loading on the cartridge.*

Cartridge Preparation

3. Place the desired number of 150 mg cartridges into the female luer ports of the manifold and collection tubes (if desired) in the rack below the output guides. *We routinely use a 12-port manifold.*
4. Turn on the vacuum and adjust the pressure to ~7mm Hg using the vacuum control valve. (If no control valve is available on your manifold, target a flow rate of about 1-2 drops per second). Condition the cartridge using 0.5mL of Acetonitrile followed by 1mL 2M TEAA. *The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the DMT-ON oligonucleotide to the resin.*

Purification Procedure

5. Apply the oligo/salt mixture to the cartridge in 1mL aliquots (collect the eluent and save in case of loading failure or error). *During the loading process, the DMT-ON oligos tend to stick to the cartridge packing material while most of the failure sequences are not retained.*
6. Wash the cartridge with 2 x 1mL of Salt Wash Solution. *This rinses away the remainder of the failure sequences from the cartridge.*
7. Rinse the cartridge with 2 x 1mL of 2% TFA. *A faint orange band may be visible while the DMT is removed from the bound, full-length oligonucleotide.*
8. Wash the cartridge with 2 x 1mL of deionized water. *This rinses away the TFA and excess salts.*
9. Place the appropriate receptacle (96 deep-well plate or sample tube) into the manifold and elute the purified oligo using 1 x 1mL 50% acetonitrile in water containing 0.5% ammonium hydroxide. *If desired a second elution can be performed to ensure full recovery, but in our experience, full recovery of the oligonucleotide is achieved in this one step.*
10. Note: The dilute ammonium hydroxide is utilized to neutralize any remaining TFA. Acidic conditions can damage oligonucleotides so it is important to keep the oligonucleotide solution basic (pH > 7.5) during either drying or storage of the eluted product. It is always safer to store oligos in a buffered solution such as 10mM Tris.HCl, 1mM EDTA, pH 8.0 (TE Buffer).
11. Determine the yield and store purified oligonucleotide lyophilized solid at -20°C. *Glen-Pak DNA purification should yield over 90% recovery of the original DMT-ON product. The actual yield depends on synthesis efficiency and the amount of failures in the oligonucleotide sample.*

50mg Glen-Pak™ DNA Purification Cartridge for 40 to 200 nmole Scale Oligos

The 50mg Glen-Pak DNA cartridge is used in the same manner as the standard sized Glen-Pak, but requires less volume for most of the protocol steps to allow for single reagent additions. The lower volume requirements begin with cleavage and deprotection at smaller scales and extend through most of the protocol to the elution step. The 50mg Glen-Pak DNA cartridge is only offered in a vacuum manifold configuration due to its intended use with higher throughput applications.

When using a vacuum manifold, adjust the pressure to ~7mm Hg using the vacuum control valve. (If no control valve is available on your manifold, target a flow rate of about 1 drop per second). Note: The contact time of the reagent additions is critical due to the smaller volumes utilized in the protocol, so reduce the vacuum setting to ensure 1 drop per second maximum if required.

While most of the volumes for the 50mg Glen-Pak are half of those that are used for the 150mg version, one must still use 2mL of the 2% TFA and a slow flow rate (maximum of 1 drop per second) to remove all of the DMT from the oligonucleotide.

Purification of DNA Oligonucleotides (DMT-ON)

Materials — *Amount Used*

Glen-Pak DNA Purification Cartridge (60-5000-96)	1
Vacuum manifold (96 well or 12-24 port SPE type, if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	0.5mL
Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	1mL
2% Trifluoroacetic Acid (TFA)/Water (60-4040-57)	2mL
Deionized Water	1mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	0.5mL

* Add 50uL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

Sample Preparation

1. Following DNA synthesis, deprotect the DMT-ON oligonucleotide in 0.5mL of ammonium hydroxide or ammonium hydroxide/methylamine (AMA) as normal. There is no need to lyophilize the deprotection solution. ***For this procedure to be successful, the synthesis must have been carried out DMT-ON. The base labile protecting groups must have been removed with either AMA or ammonium hydroxide (0.5mL).***
2. Add 0.5mL of 100 mg/mL Sodium Chloride solution to the deprotected DMT-ON oligonucleotide for a final volume of 1.0mL. ***The final salt concentration of the sample must be around 50 mg/mL for the loading of the oligo on the cartridge. Larger volumes may be loaded, but this is our suggested volume for loading on the cartridge.***

Cartridge Preparation

3. Place the desired number of 50 mg cartridges into the female luer ports of the manifold and collection tubes (if desired) in the rack below the output guides. ***We routinely use a 12-port manifold.***
4. Turn on the vacuum and adjust the pressure to ~7mm Hg using the vacuum control valve. (Note: Whether or not a control valve is available on your manifold, target a flow rate of about 1 drop per second for all reagents). Condition the cartridge using 0.5mL of Acetonitrile followed by 1.0mL 2M TEAA. ***The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the DMT-ON oligonucleotide to the resin.***

Purification Procedure

5. Apply the deprotected oligo/salt mixture to the cartridge (collect the eluent and save in case of loading failure or error). ***During the loading process, the DMT-ON oligos tend to stick to the cartridge packing material while most of the failure sequences are not retained.***
6. Wash the cartridge with 1.0mL of Salt Wash Solution. ***This rinses away the remainder of the failure sequences from the cartridge.***
7. Rinse the cartridge with 2 x 1.0mL of 2% TFA. ***A faint orange band may be visible while the DMT is removed from the bound, full-length oligonucleotide.***
8. Wash the cartridge with 1.0mL of deionized water. ***This rinses away the TFA and excess salts.***
9. Place the appropriate receptacle (96 deep-well plate or sample tube) into the manifold and elute the purified oligo using 1 x 0.5 mL 50% acetonitrile in water containing 0.5% ammonium hydroxide. ***If desired a second elution can be performed to ensure full recovery, but in our experience, full recovery of the oligonucleotide is achieved in this one step.***
10. Note: The dilute ammonium hydroxide is utilized to neutralize any remaining TFA. Acidic conditions can damage oligonucleotides so it is important to keep the oligonucleotide solution basic (pH > 7.5) during either drying or storage of the eluted product. It is always safer to store oligos in a buffered solution such as 10mM Tris.HCl, 1mM EDTA, pH 8.0 (TE Buffer).
11. Determine the yield and store purified oligonucleotide lyophilized solid at -20°C. Glen-Pak DNA purification should yield over 90% recovery of the original DMT-ON product. ***The actual yield depends on synthesis efficiency and the amount of failures in the oligonucleotide sample.***

Purification of Phosphorothioate (S-Oligo) DNA (DMT-ON)

Using the procedure below, a DMT-ON phosphorothioate modified oligonucleotide can be purified utilizing the DMT.

Materials	*Amount Used*
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	1mL
Salt Wash Solution (12% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
4% Trifluoroacetic Acid (TFA)/Water (See DNA Materials on Page 29)	2mL
Deionized Water	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL

* Add 50uL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

The purification procedure is the same as described in the DMT-ON DNA purification section on the previous pages with the following exceptions:

1. Salt wash step (6): The salt wash solution for removal of phosphorothioate, DMT-Off failure sequences contains a higher concentration of Acetonitrile (12% vs. 5%) than routinely used in standard Glen-Pak DNA purifications.

2. Trityl removal step (7): Use 2 x 1 mL additions of 4% TFA and allow the acid to flow slowly through the cartridge with a targeted contact time of 3-4 minutes ***(Note: this is a strong acid and could damage the oligonucleotide if left on the support too long; avoid interrupting the purification process during or just after this step).*** *A faint orange or yellow band may be visible while the trityl is removed from the bound, full-length oligonucleotide.*

Purification of 2'-F and 2'-OMe Modified Oligonucleotides (DMT-ON)

Using the procedure below, an oligonucleotide manufactured DMT-ON and modified with 2'-Fluoro and 2'-O-Methyl monomers can be purified on the Glen-Pak DNA cartridge utilizing the DMT.

Materials	*Amount Used*
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	1mL
Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
2% Trifluoroacetic Acid (TFA)/Water (60-4040-57)	2mL
Deionized Water	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL
Concentrated Ammonium Hydroxide	0.5mL

* Add 50uL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

The purification procedure is the same as described in the DMT-ON DNA purification section with the following exceptions:

1. Sample Preparation Step (2): After adding 1mL of 100 mg/mL sodium chloride for a final volume of 2 mL, heat the sample at 55 °C for at least 15 minutes. Proceed to step 5 in the protocol and load the sample on to a properly prepared Glen-Pak DNA cartridge.

Purification of 10-20 µmole scale DNA Oligonucleotides (DMT-ON)

The Glen-Pak™ DNA Purification Cartridge 3g (60-5300-01) offers the same, high fidelity purification as normally seen in our standard Glen-Pak DNA cartridge. The Glen-Pak 3g is a viable, cost effective alternative to HPLC, as it exploits the affinity of the DMT to purify up to 20 µmole scale syntheses in a single purification run. In addition, this is accomplished using the same number of steps and reagents as required by the standard Glen-Pak™ protocol.

Materials	*Amount Used*
Glen-Pak™ DNA Purification Cartridge 3g (60-5300-01)	1
Vacuum Manifold (12-24 port SPE type)	1
HPLC Grade Acetonitrile	10mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	20mL
100 mg/mL Sodium Chloride	10mL
Salt Wash Solution (5% Acetonitrile in 100 mg/mL Sodium Chloride)	20mL
2% Trifluoroacetic Acid (TFA)/Water (60-4040-57)	20mL
Deionized Water	20mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	

* Add 50µL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

Sample Preparation

1. Following DNA synthesis, deprotect the DMT-ON oligonucleotide in 5mL of ammonium hydroxide or ammonium hydroxide/methylamine (AMA) per 10 µmole scale synthesis. There is no need to lyophilize the deprotection solution. ***For this procedure to be successful, the synthesis must have been carried out DMT-ON. The base labile protecting groups must have been removed with either AMA or ammonium hydroxide.***
2. If purifying 2 x 10 µmole scales, add 10mL of 100 mg/mL Sodium Chloride solution to the deprotected DMT-ON oligonucleotide for a final volume of 20mL. ***The final salt concentration of the sample must be around 50 mg/mL for the loading of the oligo on the cartridge. Larger volumes may be loaded, but this is our suggested volume for loading on the cartridge.***

   Note: If you are only purifying one 10 µmole scale oligonucleotide, add another 5mL of deprotection solution for a total volume of 10mL AMA or ammonium hydroxide and add an equal volume of salt prior to purification for a total load volume of 20mL.

Cartridge Preparation

3. Place the desired number of Glen-Pak 3g cartridges into the female luer ports of the manifold and collection tubes (if desired) in the rack below the output guides. ***We routinely use a 12-port manifold.***
4. Turn on the vacuum and adjust the pressure to ~7mm Hg using the vacuum control valve. (If no control valve is available on your manifold, target a flow rate of about 1-2 drops per second). Condition the cartridge using 10mL of Acetonitrile followed by 20mL 2M TEAA. ***The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the DMT-ON oligonucleotide to the resin.***

Purification Procedure

5. Apply the oligo/salt mixture to the cartridge in 10mL aliquots (collect the eluent and save in case of loading failure or error). ***During the loading process, the DMT-ON oligos tend to stick to the cartridge packing material while most of the failure sequences are not retained.***
6. Wash the cartridge with 2 x 10mL of Salt Wash Solution. ***This rinses away the remainder of the failure sequences from the cartridge.***
7. Rinse the cartridge with 2 x 10mL of 2% TFA. ***A faint orange color may be visible while the DMT is removed from the bound, full-length oligonucleotide.***
8. Wash the cartridge with 2 x 10mL of deionized water. ***This rinses away the TFA and excess salts.***
9. Place the appropriate receptacle into the manifold and elute the purified oligo using 1 x 10mL 50% acetonitrile in water containing 0.5% ammonium hydroxide. ***If desired a second elution using an additional 5mL can be performed to ensure full recovery, but in our experience, full recovery of the oligonucleotide is achieved in this one step.***
10. **Note: The dilute ammonium hydroxide is utilized to neutralize any remaining TFA. Acidic conditions can damage oligonucleotides so it is important to keep the oligonucleotide solution basic (pH > 7.5) during either drying or storage of the eluted product. It is always safer to store oligos in a buffered solution such as 10mM Tris.HCl, 1mM EDTA, pH 8.0 (TE Buffer).**
11. Determine the yield and store purified oligonucleotide lyophilized solid at -20°C. ***Glen-Pak DNA purification should yield over 90% recovery of the original DMT-ON product. The actual yield depends on synthesis efficiency and the amount of failures in the oligonucleotide sample.***

Glen-Pak™ DNA 96-Well Plate Purification

The 96-well filter plate (60-5400-01) is standardized to fit on any vacuum-based solid phase extraction (SPE) system and can be implemented successfully with a small amount of method development. For those customers who do not already have a system in place, we have tested a vacuum collar manifold that allows use with any standard vacuum pump (see ***Equipment Information*** section below).

The advantages of using the 96-well format include:

- Lower cost processing (less labor and unattended operation if using a robot).
- Less processing time per oligo when using a whole plate.
- Less solvent usage for every purification step.
- Less final elution volume to dry in preparation for analysis or use.

The protocol below was optimized using the CaptiVac collar system from Varian (A796). The standard sample load and rinse volumes are 0.5mL with a final elution volume of 0.25mL, which is collected into a 96-well deep well plate. These volumes are only suggestions based on our experience and could be lower with proper method development. The plate should also be compatible with previously installed 96-well filter plate and SPE manifold systems.

96-Well Purification of DNA Oligonucleotides (DMT-ON)

Materials

Materials	*Amount used*
Glen-Pak DNA 96-Well Plate (60-5400-01)	1
96-Well Filter Plate Collar Vacuum manifold	1
HPLC Grade Acetonitrile	0.5mL/well
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	0.5mL/well
100 mg/mL Sodium Chloride	0.25mL/well
Salt Wash Solution (5% Acetonitrile in 100 mg/mL Sodium Chloride)	0.5mL/well
2% Trifluoroacetic Acid (TFA)/Water (60-4040-57)	0.5mL/well
Deionized Water	0.5mL/well
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	0.25mL/well
96-well deep well collection plate	1
Waste collection reservoir	1

* Add 50µL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution

Procedure

Sample Preparation

1. Following 10-50 nmole DNA synthesis, deprotect the DMT-ON oligonucleotide in 0.25mL of ammonium hydroxide or ammonium hydroxide/methylamine (AMA) as normal. There is no need to lyophilize the deprotection solution. ***For this procedure to be successful, the synthesis must have been carried out DMT-ON. The base labile protecting groups must have been removed with either ammonium hydroxide or AMA.***
2. Add 0.25mL of 100 mg/mL Sodium Chloride solution to the deprotected DMT-ON oligonucleotide for a final volume of 0.5mL. ***The final salt concentration of the sample must be around 50 mg/mL for the loading of the oligo on the cartridge. Larger and possibly smaller volumes may be loaded, but this is our suggested volume for loading on the wells.***

Glen-Pak 96-Well Plate Preparation

3. Assemble the vacuum collar, Glen-Pak DNA 96-well plate and waste collection reservoir and attach it to a vacuum source using 1/4" ID tubing. ***(Note: See Equipment Information section below for more detail).***
4. Turn on the vacuum; adjust the CaptiVac control valve knob to expose first 4 colored bands. ***(Note that the vacuum is present but not sufficient to securely hold either Waste Plate or Sample Collection Plate securely to the vacuum collar).***
5. Add 0.5mL of Acetonitrile to each well to be used. Wait 2 minutes. Add 0.5mL 2M TEAA. Wait 2 minutes. Turn off the vacuum. Note: Confirm flow during these steps. Liquid should be visible in the waste collection reservoir. Also, check each well for liquid. It should flow into the well and no longer be visible resting atop the substrate. If liquid has not passed into and through substrate, patiently turn the vacuum control knob clockwise to cover the colored bands until flow is established. ***The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the DMT-ON oligonucleotide to the resin.***

Purification Procedure

6. Turn on the vacuum as described in step 4, apply the 0.5mL oligo/salt mixture from step 2 to the plate, and wait 2 minutes. (Optionally use a 96-well deep well plate under the vacuum collar and filter plate instead of the waste collection reservoir in order to collect the eluent in case of loading failure or error). ***During the loading process, the DMT-ON oligos tend to stick to the packing material while most of the failure sequences are not retained.***
7. Add 0.5mL of Salt Wash Solution to each well and wait 2 minutes. ***This rinses away the remainder of the failure sequences from the cartridge.***
8. Add 0.5mL of 2% TFA to each well and wait 2 minutes. ***A faint orange color may be visible while the DMT is removed from the bound, full-length oligonucleotide.***
9. Wash each well with 0.5mL of deionized water. ***This rinses away the TFA and excess salts.***
10. Place a 96 deep-well plate into the manifold assembly and elute the purified oligos using 1 x 0.25mL 50% acetonitrile in water containing 0.5% ammonium hydroxide and turn off vacuum source. If desired a second elution can be performed to ensure full recovery, but in our experience, full recovery of the oligonucleotide is achieved in this one step. ***Note: The dilute ammonium hydroxide is utilized to neutralize any remaining TFA. Acidic conditions can damage oligonucleotides so it is important to keep the oligonucleotide solution basic (pH > 7.5) during either drying or storage of the eluted product. It is always safer to store oligos in a buffered solution such as 10mM Tris.HCl, 1mM EDTA, pH 8.0 (TE Buffer).***
11. Dry down the eluted oligos. Determine the yield and store purified oligonucleotides lyophilized solid at -20°C. ***Glen-Pak DNA purification should yield over 90% recovery of the original DMT-ON product. The actual yield depends on synthesis efficiency and the amount of failure sequences in the oligonucleotide sample.***

Equipment Information

- CaptiVac vacuum collar (Varian A796 or equivalent), includes the collar, 2 spare gaskets and a brass vacuum control valve that is attached to a "T" fitting.
- Waste Collection Reservoir (Nalgene Robotic Reservoir (300mL) 1200-1300 or equivalent).
- Vacuum tubing 20 inches, ¼" ID, 7/16" OD.
- A seal for unused wells in Glen-Pak 96-well plate (Glen Research, 60-0020-01) may be used to ensure a vacuum seal if you are not planning to run a full plate of oligos.
- 96-well deep well collection plates (Varian A696001000 or equivalent).

Purification of 5' Amino Modified Oligonucleotides (MMT and DMS(O)MT-ON)

Using the procedure below, an oligonucleotide labeled with 5'-Amino Modifier C6 MMT (10-1906-XX) will elute with both purified MMT-ON and purified MMT-OFF product, while purified oligos with a 5'-Amino Modifier C6 DMS(O)MT (10-1907-XX) will elute fully detritylated (see Glen Report GR19-1 pp 6-7 for more information on the performance of the various amino-modifiers during cartridge purification).

Materials — *Amount Used*

Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	1mL
Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
4% Trifluoroacetic Acid (TFA)/Water (See DNA Materials on Page 29)	2mL
Deionized Water	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL

* Add 50µL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

The purification procedure is the same as described in the DMT-ON DNA purification section with the following exceptions and taking account of the specific comments below:

1. In the past, we have noted that when the amino-modifier contains the "thermally labile" MMT protecting group, carry out the deprotection at 40°C for 24 hours. We have now shown that this is not necessary and that these trityl amines are stable to the elevated temperatures routines used for oligonucleotide deprotection.
2. When using DMS(O)MT-amino modifier (10-1907) do not concentrate the oligo before Trityl on purification. If this is necessary, add 45mg of Tris base/mL of deprotection solution.
3. Trityl removal step (7): Use 2 x 1mL additions 4% TFA and allow the acid to flow slowly through the cartridge with a targeted contact time of 5 minutes (Note: this is a strong acid and could damage the oligonucleotide if left on the support too long; DO NOT interrupt the purification process during or just after this step). ***A faint orange or yellow band may be visible while the trityl is removed from the bound, full-length oligonucleotide.***
4. As an added precaution, a second final elution with 1mL 75% Acetonitrile in 0.5% ammonium hydroxide may be used to remove any additional oligo that did not elute fully.

Purification of 5’ Biotin Modified Oligonucleotides (DMT-ON)

Using the procedure below, an oligonucleotide labeled with 5’-Biotin (10-5950-XX) or 5’ Biotin-TEG (10-1955-XX) can be purified utilizing a retained DMT.

Materials	*Amount Used*
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	1mL
Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
2% Trifluoroacetic Acid (TFA)/Water (60-4040-57)	2mL
Deionized Water	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL

* Add 50µL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

The purification procedure is the same as described in the DMT-ON DNA purification section with one exception. 5’-Biotin is slow to detritylate. When the final DMT-group is left on to aid in cartridge purification, leave the oligo in contact with the TFA solution for 10 minutes.

Purification of 5’ CPR II Modified Oligonucleotides (DMT-ON)

Using the procedure below, an oligonucleotide manufactured DMT-ON and modified with 5’-Phosphate using CPR II (10-1901-XX) or Solid CPR II (10-1902-XX) can be purified utilizing the DMT.

Materials	*Amount Used*
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	1mL
Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
4% Trifluoroacetic Acid (TFA)/Water (See DNA Materials on Page 29)	2mL
Deionized Water	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL
Concentrated Ammonium Hydroxide	0.5mL

* Add 50µL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

The purification procedure is the same as described in the DMT-ON DNA purification section with the following exceptions:

1. Trityl removal step (7): Use 2 x 1mL additions of 4% TFA and allow the acid to flow slowly through the cartridge with a targeted contact time of 3-4 minutes. (Note: this is a strong acid and could damage the oligonucleotide if left on the support too long; avoid interrupting the purification process during or just after this step). ***A faint orange or yellow band may be visible while the trityl is removed from the bound, full-length oligonucleotide.***

2. For CPR II, after the elution step, add 0.5mL of concentrated ammonium hydroxide to the elution volume mix well and incubate at room temperature for 20 minutes. ***This completes the elimination of the side chain to the 5’ phosphate.***

3. For Solid CPR II, dry the oligonucleotide down and use one of the following deprotection conditions to complete the elimination to yield the 5’-phosphate.

    a) 30% Ammonium hydroxide for 2 hours at 55 °C
    b) AMA (30% Ammonium hydroxide/40%Methylamine 1:1 v/v) for 10 minutes at 65 °C
    c) 0.1 M NaOH at room temperature for 5 minutes.

    This completes the elimination of the side chain to the 5’ phosphate.

Purification and Reduction of Thiol-Modified Oligonucleotides (5'-C6 S-S, 10-1936)

The three-step protocol below can be used to purify, reduce, and desalt a DMT-ON oligonucleotide labeled with 5'-Thiol-Modifier C6 S-S (10-1936). Following all three steps of the protocol below yields a functional, thiol modified oligonucleotide ready for conjugation. Please note, the procedure requires two Glen-Pak DNA cartridges but the oligo can be stored in its unreduced form DMT-ON after the first purification, if so desired. One notable difference in this procedure from many of the others is the lack of any DMT removal using TFA.

Purification, Reduction and Desalting of 5' Thiol-Modified DNA (DMT-ON)

Materials	*Amount Used*
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	2
Vacuum manifold (96 well or 12-24 port SPE type, if appropriate)	1
HPLC Grade Acetonitrile	1.1mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	2mL
0.1M Triethylamine Acetate, pH7	7mL
100 mg/mL Sodium Chloride	1mL
Salt Wash Solution (5% Acetonitrile in 100mg/mL Sodium Chloride)	2mL
Deionized Water	4mL
50% Acetonitrile/Water	1mL
10% Acetonitrile/Water	1mL
0.2M DTT (dithothreitol) in 0.1M phosphate buffer, pH=8.3-8.5	1mL

Procedure

Procedure

Sample Preparation

1. Following DNA synthesis, deprotect the DMT-ON oligonucleotide in 1.0 mL of ammonium hydroxide/methylamine (AMA) as normal. There is no need to lyophilize the deprotection solution. ***For this procedure to be successful, the synthesis must have been carried out DMT-ON. The base labile protecting groups must have been removed with either AMA or concentrated Ammonium Hydroxide (1.0mL).***
2. Add 1mL of 100 mg/mL Sodium Chloride solution to the deprotected DMT-ON oligonucleotide for a final volume of 2.0mL. ***The final salt concentration of the sample must be around 50 mg/mL for the loading of the oligo on the cartridge. Larger volumes may be loaded, but this is our suggested volume for loading on the cartridge.***

Cartridge Preparation

1. Place the desired number of 150 mg cartridges into the female luer ports of the manifold and collection tubes (if desired) in the rack below the output guides. ***We routinely use a 12-port manifold.***
2. Turn on the vacuum and adjust the pressure to ~7mm Hg using the vacuum control valve. (If no control valve is available on your manifold, target a flow rate of about 1-2 drops per second). Condition the cartridge using 0.5mL of Acetonitrile followed by 1.0mL 2M TEAA. ***The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the DMT-ON oligonucleotide to the resin.***

DMT-ON Oligo Purification Procedure

1. Apply the oligo/salt mixture to the cartridge in 1.0mL aliquots (collect the eluent and save in case of loading failure or error). ***During the loading process, the DMT-ON oligos tend to stick to the cartridge packing material while most of the failure sequences are not retained.***
2. Wash the cartridge with 2 x 1.0mL of Salt Wash Solution. ***This rinses away the remainder of the failure sequences from the cartridge.***
3. Wash the cartridge with 2 x 1.0mL of deionized water. ***This rinses away the excess salts.***
4. Place the appropriate receptacle (96 deep-well plate or sample tube) into the manifold and elute the purified, DMT-ON oligo using 1 x 1.0mL 50% acetonitrile in water. ***If desired a second elution can be performed to ensure full recovery, but in our experience, full recovery of the oligonucleotide is achieved in this one step.***
5. Note: Acidic conditions can damage oligonucleotides so it is important to keep the oligonucleotide solution basic (pH > 7.5) during either drying or storage of the eluted product. It is always safer to store oligos in a buffered solution such as 10mM Tris.HCl, 1mM EDTA, pH 8.0 (TE Buffer).
6. To keep the oligo in its DMT-ON, unreduced form, determine the yield and store purified oligonucleotide in the elution solution at -20°C. ***Glen-Pak DNA purification should yield over 90% recovery of the original DMT-ON product. The actual yield depends on synthesis efficiency and the amount of failures in the oligonucleotide sample. This elution can be used directly in the disulfide cleavage step below.***

Disulfide cleavage step (reduction done off cartridge using product from step 6 above)

1. Add an equal volume of 0.2M DTT (dithothreitol) in 0.1M phosphate buffer, pH 8.3-8.5, to the purified DMT-ON product isolated in step 10 above.
2. Incubate the solution for 30 minutes at Room Temperature. ***This reduces the disulfide and therefore separates the DMT from the oligonucleotide.***

5' Thiol-Modified Oligo Desalting Step.

1. Condition the desired number of Glen-Pak DNA cartridges as instructed in the Cartridge Preparation section above (Acetonitrile followed by TEAA).
2. Dilute the reduction reaction mixture with 5mL of 0.1M TEAA, pH 7 for a final volume of ~ 7mL. ***Assuming a full, 2mL reduction reaction, this dilutes the acetonitrile content of the reaction mixture to < 6%.***
3. Apply the 7mL of oligonucleotide mixture to the properly prepared cartridge in 1.0mL aliquots (collect the eluent and save in case of loading failure or error).
4. Wash the cartridge with 2 x 1.0mL of 0.1M TEAA, pH 7.
5. Wash the cartridge with 2 x 1.0mL of deionized water. ***This rinses away the excess salts.***
6. Elute the purified, desalted, thiol modified oligonucleotide in 10% Acetonitrile/Water. ***This elutes the oligo and leaves behind the DMT-S linkage removed during the disulfide cleavage step.***
7. Dry down the reduced thiol and bring up in the conjugation buffer of your choice. Please refer to the technical bulletin for this product for the general labeling procedure.

   http://www.glenresearch.com//Technical/TB_Thiol_Modifier_S-S.pdf

Desalting of Oligonucleotides

The DNA Glen-Pak cartridges can be used for desalting DNA or RNA oligonucleotides directly after deprotection or post purification by HPLC and Polyacrylamide Gel Electrophoresis (PAGE). The cartridges are designed specifically for DMT-ON purification where failure sequences not containing a 5' DMT are eluted with salt washes, but when an oligo is loaded in 0.1M TEAA instead of 100mM sodium chloride, a DMT-Off oligo may also be captured on the column. As with other SPE methods, it is suggested that the oligo be applied to the cartridge in an aqueous solution or one containing less than 5% organic solvent. It is always prudent to keep loading and rinse volumes until the purified product is quantified. Of special note is that the DMT-Off method for crude, deprotected oligos below uses the Glen-Pak DNA cartridge for desalting of BOTH RNA and DNA oligonucleotides. This will allow our customers currently using more than one cartridge platform for downstream processing to harmonize to only one column type for DMT-Off desalting.

Materials

Materials	Amount Used
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
0.1M TEAA, pH7	as needed
Deionized Water (RNase Free for use with RNA oligonucleotides)	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL
10% Acetonitrile/Water (for desalting of crude RNA and DNA)	1mL

* Add 50uL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

Sample Preparation

1. HPLC purified oligos: Add the required amount of 0.1M TEAA to the post purification sample to obtain ≤ 5% final concentration of acetonitrile.
2. PAGE purified oligos: Elute purified oligonucleotide from the gel slices in 0.05M TEAA as described in current protocols, filter away gel slices and bring up to at least 2mL in 0.1M TEAA.
3. DMT-Off, crude, deprotected oligos in NH4OH or AMA: Evaporate the deprotected oligo to dryness and reconstitute in 2mL 0.1M TEAA.

Cartridge Preparation

4. Prepare the cartridge as described for DMT-ON purification.

Desalting Procedure HPLC Purified Oligos:

5. Load the solution containing the oligonucleotide onto the cartridge.
6. Flush the cartridge with 2 x 1 mL of deionized water. ***This wash removes the salts from the cartridge.***
7. Elute the desalted oligonucleotide by flushing the cartridge with 1mL 50% Acetonitrile/ water containing 0.5% ammonium hydroxide. ***The product should elute fully in 1mL of 50% Acetonitrile with 0.5% ammonium hydroxide.***

Desalting Procedure PAGE Purified Oligos:

8. Load the solution containing the oligonucleotide onto the cartridge.
9. Flush the cartridge with 2 x 1mL of deionized water. ***This wash removes the salts from the cartridge.***
10. Elute the desalted oligonucleotide by flushing the cartridge with 1mL 50% Acetonitrile/ water containing 0.5% ammonium hydroxide. ***The product should elute fully in 1mL of 50% Acetonitrile with 0.5% ammonium hydroxide.***

Desalting Procedure Crude Deprotected DNA Oligos:

11. Load the solution containing the oligonucleotide onto the cartridge.
12. Flush the cartridge with 2 x 1mL of 0.1M TEAA.
13. Flush the cartridge with 2 x 1mL deionized water. ***This wash removes the salts from the cartridge.***
14. Elute the desalted oligonucleotide by flushing the cartridge with 1mL 10% Acetonitrile/ water. ***The product should elute fully in 1mL while leaving behind organics such as benzamide on the column matrix.***

Desalting Procedure Crude Deprotected RNA Oligos:

15. Conduct 2' deprotection and quenching of DMT-Off RNA oligonucleotides as suggested in the technical bulletins for TBDMS and TOM RNA monomers.
16. Load the resultant 2mL of deprotected/quenched RNA solution directly on a Glen-Pak DNA cartridge that has been prepared using the same methods as above.
17. Rinse with 2.0mL 0.1M TEAA (Fresh 2.0M TEAA diluted in RNase free water)
18. Rinse with 2.0mL RNase free water.
19. Elute the desalted product in 10% Acetonitrile in RNase free water

Oligonucleotide Storage:

20. Note: Acidic conditions can damage oligonucleotides so it is important to keep the oligonucleotide solution basic (pH > 7.5) during either drying or storage of the eluted product. It is always safer to store oligos in a buffered solution such as 10mM Tris.HCl, 1mM EDTA, pH 8.0 (TE Buffer).
21. Determine the yield and store purified oligonucleotide lyophilized solid at -20°C. ***Glen-Pak desalting should yield over 90% recovery of the original product.***

Purification of 5' Cyanine Dye and Dabcyl/Dabsyl Quencher Oligonucleotides

Follow the synthesis and deprotection guidelines for the label(s) of choice and remove any 5' protection such as the MMT or DMT. Our 6-Fluorescein phosphoramidite (10-1964-XX) has a DMT group and we recommend following the DMT-ON purification protocol above. Most of the dye and quencher labeled oligonucleotides can be deprotected at room temperature in concentrated ammonium hydroxide for a time appropriate for the monomers used. The ammonia deprotection solution can be loaded on the column directly after dilution with 100mg/mL sodium chloride as described below. Appropriate catalog items for this section are as follows:

5'-Dabcyl (10-5912-XX), 5'-Cyanine 3(10-5913-XX), 5'-Cyanine 3.5 (10-5914-XX), 5'-Cyanine 5 (10-5915-XX), 5'-Cyanine 5.5 (10-5916-XX)

A procedure on the following pages is used for oligos containing Cyanine 5 and Cyanine 5.5 deprotected using ammonium hydroxide.

Materials

Materials	Amount Used
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
100 mg/mL Sodium Chloride	3mL
Deionized Water	2mL
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL

* Add 50µL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

Sample Preparation

1. Following synthesis, deprotect the dye/quencher-ON oligonucleotide in 1mL of ammonium hydroxide as normal. There is no need to lyophilize the deprotection solution. ***For this procedure to be successful the Oligonucleotide, must not have any trityl groups on the 5' end and the base labile protecting groups must have been removed.***

2. Add 1mL of 100 mg/mL Sodium Chloride solution to the deprotected oligonucleotide for a final volume of 2mL. ***The final salt concentration of the sample must be around 50 mg/mL for the loading of the oligo on the column. Larger volumes may be loaded, but this is our suggested volume for loading on the cartridge.***

Cartridge Preparation

3. Place the desired number of 150 mg cartridges into the female luer ports of the manifold and collection tubes (if desired) in the rack below the output guides. ***We routinely use a 12-port manifold.***

4. Turn on the vacuum and adjust the pressure to ~7mm Hg using the vacuum control valve (if no control valve is available on your manifold, target a flow rate of about 1-2 drops per second). Condition the cartridge using 0.5mL of Acetonitrile followed by 1mL 2M TEAA. ***The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the dye/quencher-ON oligonucleotide to the resin.***

Purification Procedure

5. Apply the oligo/salt mixture to the cartridge in 1mL aliquots (collect the eluent and save in case of loading failure or error). ***During the loading process, the dye/quencher-ON oligos tend to stick to the cartridge packing material while the failure sequences do not.***

6. Wash the cartridge with 2 x 1mL of 100mg/mL sodium chloride. ***This rinses away the remainder of the failure sequences from the cartridge.***

7. Wash the cartridge with 2 x 1mL of deionized water. ***This rinses away excess salts. There may be some minimal breakthrough of dye-coupled oligonucleotide in this step, but in our experience, the loss is minimal.***

8. Place the appropriate receptacle (96 deep-well plate or sample tube) into the manifold and elute the purified oligo using 1 x 1mL 50% acetonitrile in water with 0.5% ammonium hydroxide. ***Use the color of the product to determine the extent of the elution from the cartridge.***

9. Determine the yield and store purified oligonucleotide lyophilized solid at -20°C. ***Glen-Pak DNA purification should yield over 90% recovery of the original dye/quencher-ON product. The actual yield depends on synthesis efficiency, dye integrity post deprotection and the amount of failures in the oligonucleotide sample.***

Purification of Cyanine 5 and Cyanine 5.5 DNA Oligonucleotides Containing Dye Degradation Products

We routinely recommend use of UltraMild phosphoramidites when synthesizing oligonucleotides containing Cyanine 5 and Cyanine 5.5, as the dye is very sensitive to the ammonium hydroxide and heat normally utilized in deprotection. That said; sometimes, other sequence and chemistry requirements dictate the use of concentrated ammonium hydroxide at room temperature leading to the production of a yellow chromophore, which is the degradation product of the Cyanine 5. One can tell significant degradation has occurred when the solution goes from a clean blue color to turquoise or green. The purification protocol below may be used to separate the degradation products from the remaining viable Cy dye labeled oligonucleotide. The appropriate catalog items for this section are as follows: Cyanine 5 (10-5915-XX), Cyanine 5.5 (10-5916-XX)

Materials

Materials	Amount Used
Glen-Pak DNA Purification Cartridge (60-5100-XX, 60-5200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
0.1M TEAA, pH7	as needed
16% Acetonitrile in 0.1M TEAA	
50% Acetonitrile/Water containing 0.5% ammonium hydroxide*	1mL

* Add 50uL of concentrated Ammonium Hydroxide per 10mL of 50% Acetonitrile solution.

Procedure

Sample Preparation

1. Following synthesis, deprotect the Cyanine 5 or Cyanine 5.5 labeled oligonucleotide in 1.0 mL of ammonium hydroxide at room temperature. Lyophilize the deprotection solution. ***For this procedure to be successful the Oligonucleotide, must not have any trityl groups on the 5' end and the base labile protecting groups must have been removed.***

2. Reconstitute the oligonucleotide in 2mL of 0.1M TEAA, pH7. ***Larger volumes may be loaded, but this is our suggested volume for loading on the cartridge.***

Cartridge Preparation

3. Place the desired number of 150 mg cartridges into the female luer ports of the manifold and collection tubes (if desired) in the rack below the output guides. ***We routinely use a 12-port manifold.***

4. Turn on the vacuum and adjust the pressure to ~7mm Hg using the vacuum control valve (if no control valve is available on your manifold, target a flow rate of about 1-2 drops per second). Condition the cartridge using 0.5mL of Acetonitrile followed by 1.0mL 2M TEAA. ***The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the dye/quencher-ON oligonucleotide to the resin.***

Purification Procedure

5. Apply the oligo/TEAA mixture to the cartridge in 1.0mL aliquots (collect the eluent and save in case of loading failure or error). ***During the loading process, the dye/quencher-ON oligos tend to stick to the cartridge packing material.***

6. Wash the cartridge with 2 x 1.0mL of 0.1M TEAA, pH7. ***This rinses away various organics and further binds the backbone of the oligonulceotide to the cartridge.***

7. Wash the cartridge with 2 x 1.0mL of 16% Acetonitrile in 0.1M TEAA. ***This rinses away the failure sequences from the synthesis and removes the Cyanine 5/5.5 degradation products. There may be some breakthrough of intact dye-coupled oligonucleotide in this step, but in our experience, the loss is minimal.***

8. Place the appropriate receptacle (96 deep-well plate or sample tube) into the manifold and elute the purified oligo using 1 x 1.0mL 50% acetonitrile in water with 0.5% ammonium hydroxide. ***Use the color of the product to determine the extent of the elution from the cartridge.***

9. Determine the yield and store purified oligonucleotide lyophilized solid at -20°C. ***Glen-Pak DNA purification should yield over 90% recovery of the original dye/quencher-ON product. The actual yield depends on synthesis efficiency, dye integrity post deprotection and the amount of failures in the oligonucleotide sample.***

Purification of 5’ 6-FAM, HEX and TET- Labeled Oligonucleotides

These popular dyes are not hydrophobic enough to be purified using the 5’ Dye-ON procedure above. Our 6-Fluorescein phosphoramidite (10-1964-XX) has a DMT group and we recommend following the DMT-ON purification protocol above. Note also that the Poly-Pak cartridges are viable alternatives for cartridge purification of oligos containing these labels. The Dye-ON Procedure can be found in the Poly-Pak Booklet:

http://www.glenresearch.com/Technical/PolyPakBooklet.pdf

Glen-Pak RNA Cartridge Purification (DMT-ON)

The procedure below is detailed for use with both TOM and TBDMS protected RNA phosphoramidites. For further information on RNA synthesis, deprotection, and purification, please reference Glen Reports 19-2 December 2007 and 21-1 May 2009.

Materials	*Amount Used*
Glen-Pak RNA Purification Cartridge (60-6100-XX, 60-6200-XX)	1
Vacuum manifold (96 well or 12-24 port SPE type if appropriate)	1
Oven or dry block for 65ºC incubation	1
Evaporation apparatus, house air or Speed Vac System	1
50:50 ammonium hydroxide/40% aqueous methylamine (AMA)	2mL
TEA.3HF de-silylation solution (see RNA Materials section below)	250µL
Glen-Pak RNA Quenching Buffer (60-4120-XX)	1.75mL
HPLC Grade Acetonitrile	0.5mL
2.0M Triethylamine Acetate (60-4110-XX, TEAA, pH7)	1mL
10% Acetonitrile, 90% 2M TEAA, pH 7	1mL
2% Trifluoroacetic Acid (TFA)/Water (60-4040-57)	2mL
RNase Free Water	3mL
1M ammonium bicarbonate/30% Acetonitrile	1mL

Required Synthesis and Deprotection Procedure

Synthesis and Base Deprotection

1. When using TBDMS protected RNA amidites, manufacture the RNA DMT-ON using Acetyl-C-CE phosphoramidite (we use Glen Research’s TBDMS Bz-A, Ac-G, Ac-C and U phosphoramidites). ***Acetyl protection on the C RNA monomer allows for Ammonia/Methylamine (AMA) deprotection while the DMT is required for purification of the full-length product.*** Please refer to our latest TBDMS Technical Bulletin which can be found here: http://www.glenresearch.com//Technical/TB_TBDMS.pdf

2. When using TOM protected RNA amidites, manufacture the RNA DMT-ON following the procedure in the TOM Technical bulletin which can be found here: http://www.glenresearch.com//Technical/TB_TOM.pdf

3. WARNING: DO NOT USE heat when drying down your DMT-ON RNA post base deprotection to avoid loss of DMT.

    a. Using heat may remove your DMT and it will not adhere to the cartridge.
    b. Ideally, blow an air source such as nitrogen or house air over the oligonucleotide deprotection solution to dry down the sample. An evaporator system may also be used.
    c. Some speed-vac systems may also remove DMT from the oligonucleotide.

2’ Deprotection of the RNA Oligonucleotide (see RNA Materials section below)

Note: When Glen-Pak purifying TOM RNA, **DO NOT use TBAF** for 2’ deprotection of your RNA as suggested in our older TOM Technical Bulletins. Use the 2’ Deprotection cocktail as described in this Glen-Pak RNA Cartridge purification section and the newest bulletin referenced above.

4. Fully dissolve the RNA oligonucleotide in 115µL DMSO. If necessary, heat the oligo at 65C for about 5 minutes to get it into solution. Make sure the oligonucleotide is in solution before proceeding to the next step.

5. Add 60µL of TEA to the DMSO/oligo solution and mix gently. ***This keeps the solution at a***

basic pH, thus protecting the DMT group on the 5' end of the oligonucleotide.

6. Finally, add 75µL of TEA.3HF and mix gently. *This is the reagent that desilylates the RNA.*

7. Heat the mixture at 65C for 2.5 hours. *This reaction removes the TBDMS, 2' protection and yields a fully functional RNA oligo while maintaining the DMT for use in purification on the Glen-Pak RNA cartridge.*

RNA Purification Procedure

Sample Preparation

8. Immediately before cartridge purification is to begin, cool the sample and add 1.75mL of Glen-Pak RNA Quenching Buffer to the deprotected RNA solution. Mix well. *This buffer quenches the de-silylation reaction and preps the RNA for loading on the Glen-Pak RNA cartridge.*

Cartridge Preparation

9. Place the desired number of cartridges into the female luer ports of the manifold and collection tubes (if desired) in the rack below the output guides. *We routinely use a 12-port manifold.*

10. Turn on the vacuum and adjust the pressure to ~7mm Hg using the vacuum control valve. (If no control valve is available on your manifold, target a flow rate of about 1-2 drops per second). Condition the cartridge using 0.5mL of Acetonitrile followed by 1.0mL 2M TEAA. *The acetonitrile washes organic residues from the resin and wets it, while the TEAA acts as an ion-pairing reagent to enhance the binding of the DMT-ON oligonucleotide to the resin.*

Purification Procedure

11. Apply the RNA/Quenching Buffer mixture to the cartridge in 1mL aliquots. (Collect the eluent and save in case of loading failure or error). *During the loading process, the DMT-ON oligos tend to stick to the cartridge packing material while most of the failure sequences are not retained.*

12. Wash the cartridge with 1mL of 10% Acetonitrile, 90% 2M TEAA, pH 7.0. *This rinses away the remainder of the failure sequences from the cartridge.*

13. Wash the cartridge with 1mL of RNase Free water. This rinses away excess salts.

14. Rinse the cartridge with 2 x 1mL of 2% TFA. *TFA is a strong acid and could damage the oligonucleotide if left on the support too long; it is not recommended to interrupt the purification process during or just after this step.*

15. Wash the cartridge with 2 x 1mL of RNase Free water. *This rinses away excess TFA.*

16. Place the appropriate sterile receptacle (96 deep-well plate or sample tube) into the manifold and elute the purified oligo using 1 x 1mL 1M ammonium bicarbonate/30% Acetonitrile. *Ammonium bicarbonate is a volatile salt so the RNA can now be dried down in a Speed Vac and brought up in your buffer of choice.*

RNA Materials

A. Phosphoramidites

1. TBDMS RNA Phosphoramidites
   a. 10-3003-XX, Bz-A
   b. 10-3015-XX, Ac-C
   c. 10-3025-XX, Ac-G
   d. 10-3030-XX, U
2. TOM Protected RNA Phosphoramidites
   a. 10-3004-XX, A
   b. 10-3014-XX, C
   c. 10-3024-XX, G
   d. 10-3034-XX, U

B. TEA.3HF de-silylation solution

1. 115µL DMSO: Dimethylsulfoxide, anhydrous, 99.9% (eg. Aldrich 276855)
2. 60µL TEA: Triethylamine, puriss. p.a. ≥ 99.5% (GC)
3. 75µL TEA.3HF: Triethylamine trihydrofluoride, 98% (eg. Aldrich 34,464-8)

C. 10% Acetonitrile, 90% 2M TEAA, pH 7.0 (100mL)

1. 10mL HPLC grade Acetonitrile
2. 90mL 2M TEAA, pH 7.0 (60-4110-XX)

D. 30% Acetonitrile in 1M ammonium bicarbonate (33mL, 0.7M final concentration)

1. 1.82g Ammonium Bicarbonate
2. 23.1 mL RNase Free water
3. 9.9 mL HPLC grade Acetonitrile
   a. Weigh the Ammonium Bicarbonate into a sterile, 50mL conical tube.
   b. Add the RNase Free water and fully dissolve the Ammonium Bicarbonate.
   c. Add the Acetonitrile and mix well before use.

DNA Materials

A. 4% Trifluoroacetic Acid (TFA)/Water

1. 4 mL Trifluoroacetic Acid (eg. Sigma-Aldrich T6508)
2. HPLC Grade Water or equivalent.
   a. Add 4mL of trifluoroacetic acid to a clean 100mL glass bottle with a Teflon lined cap.
   b. Dilute with 96mL of HPLC grade water.
   c. Mix well and label appropriately.
   d. Protect from light. Good for at least one year at room temerature.

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