Products for DNA Research

User Guide to Glen Gel-Pak™ Purification

GLEN RESEARCH

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Contents

Principles of Glen Gel-Pak™ for DNA/RNA Desalting

The principle of the Glen Research Gel-Pak column is based on size exclusion chromatography that separates molecules based on the hydrodynamic volume of the molecule in aqueous solutions. In gel filtration, the mobile phase is an aqueous solution and the stationary phase is a porous resin. The pores of the resin are sized such that they allow small molecules to enter the pores, yet exclude larger molecules from the pores. The small molecules, such as salts and hydrolyzed protecting groups, diffuse into the pores of the resin and move slower through the column. The larger molecules, such as DNA or proteins, are excluded from the pores and move quickly through the column. The end result is that the larger molecules elute first in the column void volume while the small molecules are still flowing through resin of the column.

The Glen Gel-Pak columns are ideal for desalting and clean up of conjugation reactions. They can be used for removal of the ammonium hydroxide deprotection solution and the hydrolyzed protecting groups after deprotection. The columns can also be used for the clean up of NHS-labeling reactions to separate the labeled oligo and unlabeled oligo from the unreacted NHS ester, hydrolyzed label, and n-hydroxysuccimide thereby greatly simplifying the downstream purification steps.

Glen Gel-Pak columns are easy to use and available in three sizes for different sample volumes.

There are many benefits to the Glen Gel-Pak.

Versatility:

- Ability to directly desalt oligonucleotides deprotected in either 30% ammonium hydroxide OR 50:50 ammonium hydroxide/40% aqueous methylamine (AMA)
- Easily exchange buffers
- Simple clean-up of labeling reactions
- Mild method for purification from salts and solvents such as DMSO and DMF

Capacity:

- Multiple column sizes (0.2mL, 1.0mL, and 2.5mL) are available to match synthesis scale
- Ability to efficiently desalt short and long at different scales using the same protocol
- Suitable for oligos >10mer in length

Protocol for Glen Gel-Pak™ 0.2 Desalting Column

Materials	Amount Used
Glen Gel-Pak Column (61-5002-XX)	1
Aqueous Buffer, Stock Solution[1]	5.5mL

[1]The choice of buffer is determined by the downstream application. (e.g., 10mM TE buffer).

Procedure

1. Column Preparation

Remove the cap from the top of the column first and then remove the bottom cap from the column. Allow the solution to drain by gravity into a waste container until the flow stops. *(This step removes the storage buffer).*

2. Column Equilibration

Flush the column with 5mL of buffer. Allow buffer to flow by gravity. *(This step thoroughly rinses the resin).*

3. Sample Application

Load 200µl of sample solution onto the top frit of the column and allow the sample to flow into the column bed[2]. Add 150µL of buffer and allow the buffer to flow into the bed. *(This step loads the sample onto the column).*

4. Elution

Replace the waste container with a clean sample collection tube. Add 350µL of the buffer to the column to elute the sample. *(This step elutes the oligo while the salt is still on the column).*

[2]Different sample and elution volumes can be used. The sample and pre-elution volumes should follow the recommendations in the table below.

Sample Volume	Pre-elution Volume	Elution Volume
150µL	200µL	300µL
200µL	150µL	350µL
250µL	100µL	400µL
300µL[3]	50µL	450µL

[3]Although satisfactory results can be obtained with this volume, we recommend using a larger column or splitting the sample into two when working with this volume.

Protocol for Glen Gel-Pak™ 1.0 Desalting Column

Materials	Amount Used
Glen Gel-Pak Column (61-5010-XX)	1
Aqueous Buffer, Stock Solution[1]	18mL

[1]The choice of buffer is determined by the downstream application. (e.g., 10mM TE buffer).

Procedure

1. Column Preparation

Remove the cap from the top of the column first and then remove the bottom cap from the column. Allow the solution to drain by gravity into a waste container until the flow stops. *(This step removes the storage buffer).*

2. Column Equilibration

Flush the column with 15mL of buffer. Allow buffer to flow by gravity. *(This step thoroughly rinses the resin).*

3. Sample Application

Load 1000µL of sample solution onto the top frit of the column and allow the sample to flow into the column bed[2]. *(This step loads the sample onto the column).*

4. Elution

Replace the waste container with a clean sample collection tube. Add 1500µL of the buffer to the column to elute the sample. *(This step elutes the oligo while the salt is still on the column).*

[2]Smaller sample and elution volumes can be used. The sample and pre-elution volumes should follow the recommendations in the table below.

Sample Volume	Pre-elution Volume	Elution Volume[3]
300µL	1200µL	750µL
400µL	1100µL	750µL
500µL	1000µL	850µL
750µL	750µL	1000µL
1000µL	500µL	1000µL

[3]Users may need to optimize elution volumes for their specific application to improve salt removal or overall yield.

Protocol for Glen Gel-Pak™ 2.5 Desalting Column

Materials	Amount Used
Glen-Desalt Column (61-5025-XX)	1
Aqueous Buffer, Stock Solution[1]	29mL

[1]The choice of buffer is determined by the downstream application. (e.g., 10mM TE buffer).

Procedure

1. Column Preparation

Remove the cap from the top of the column first and then remove the bottom cap from the column. Allow the solution to drain by gravity into a waste container until the flow stops. *(This step removes the storage buffer).*

2. Column Equilibration

Flush the column with 25mL of buffer. Allow buffer to flow by gravity. *(This step thoroughly rinses the resin).*

3. Sample Application

Load 2500µL of sample solution onto the top frit of the column and allow the sample to flow into the column bed[2]. *(This step loads the sample onto the column).*

4. Elution

Replace the waste container with a clean sample collection tube. Add 2750µL of the buffer to the column to elute the sample. *(This step elutes the oligo while the salt is still on the column).*

[2]Smaller sample and elution volumes can be used. The sample and pre-elution volumes should follow the recommendations in the table below.

Sample Volume	Pre-elution Volume	Elution Volume[3]
1000µL	1500µL	1750µL
1500µL	1000µL	2000µL
2000µL	500µL	2250µL
2500µL	0µL	2750µL

[3]Users may need to optimize elution volumes for their specific application to improve salt removal or overall yield.

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