PD MidiTrap™ G-25

Instructions for Use

cytiva.com

28922530 AE

Table of Contents

1 Introduction

Product code

28918008

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

WARNING

WARNING: The column storage solution, 0.15% Kathon GG/ICP biocide, is potentially allergenic. Use gloves when discarding the storage solution.

2 Description

The product contains:

- 50 disposable PD MidiTrap™ columns prepacked with 3.5 mL of Sephadex™ G-25 resin
- 4 adapters

PD MidiTrap G-25 columns are designed for rapid and convenient sample cleanup of proteins and other large biomolecules (> 5000 M_r). The columns can be used in a wide range of applications such as desalting, buffer exchange, and removal of low molecular weight compounds.

The chromatography technique is gel filtration and molecules are separated on the basis of differences in size.

- Molecules larger than the largest pores in the Sephadex matrix are excluded from the matrix and eluted first, in or just after the void volume. The void volume is the column volume outside the Sephadex matrix.
- Molecules smaller than the largest pores in the Sephadex matrix will penetrate the pores to a varying extent. They have a larger accessible column volume than the large molecules and therefore they elute after the large molecules just before one total column volume of buffer has passed through the column.

Group separation can be performed using two different protocols: gravity protocol or spin protocol.

Cytiva provides an assortment of sample cleanup products. The different formats available are summarized in the table below.

Table 1. Product overview

Cleanup product	Exclusion limit, M_r	Bed volume	Sample volume, gravity protocol[1]	Sample volume, spin protocol[1]
PD SpinTrap™ G-25	5000	0.5 mL	-	100 to 180 µL
PD MultiTrap™ G-25	5000	0.5 mL	-	70 to 130 µL
PD MiniTrap™ G-25	5000	2.1 mL	0.1 to 0.5 mL	0.2 to 0.5 mL
PD MidiTrap G-25	5000	3.5 mL	0.5 to 1.0 mL	0.75 to 1.0 mL
PD-10 Desalting Columns	5000	8.3 mL	1.0 to 2.5 mL	1.75 to 2.5 mL
PD MiniTrap G-10	700	2.1 mL	0.1 to 0.3 mL	-
PD MidiTrap G-10	700	5.3 mL	0.4 to 1.0 mL	-

[1] Recommended sample volumes.

3 Advice on handling

Protocol selection

Separation can be performed using two different protocols: gravity protocol or spin protocol, see *Table 2, on page 6*.

Gravity protocol

The liquid passes through the column by gravity force.

- There is a slightly higher recovery and desalting capacity using gravity protocol compared to when using the spin protocol.
- The sample applied is diluted.

Spin protocol

Additional gravity force is added by spinning the column in a centrifuge for some protocol steps.

- The sample applied is not diluted.

Table 2. Protocol overview

Protocol	Sample volume	Elution buffer	Dilution factor	Desalting capacity
Gravity	0.5 to 1.0 mL	1.5 mL	1.5 times[1]	> 98%
Spin	0.75 to 1.0 mL	None	None	> 98%

[1] Two times dilution valid if 1.0 mL of sample volume is used.

Recovery

The recovery of the amount of sample applied is dependent on the type of protein or other biomolecule. Typically, the recovery is 70% to 90%. An increase in sample concentration can improve recovery.

Equilibration

- It is critical to equilibrate the column to remove the storage solution completely. Follow the protocol to make sure that an equilibration volume corresponding to three packed bed volumes is used.
- Equilibration is most conveniently performed by gravity also when using the spin protocol.

Sample application

- The MidiTrap column is intended for sample volumes up to 1.0 mL.

- For sample volumes less than 1.0 mL, allow the sample to enter the packed bed completely and then add equilibration buffer (stacker volume) so that the total volume of sample and buffer added equals 1.0 mL.
- Allow the sample to enter the packed bed completely before any addition of buffer for elution.

Elution

- **Only for spin control:** Use stacker volumes for higher recoveries.

Centrifugation

- **Only for spin control:** Place the columns in the same direction during all centrifugation steps for better results using a fixed-angle rotor centrifuge.

4 Column assembly

For use in the spin protocol the PD MidiTrap G-25 column must be assembled with an adapter and a collection tube as shown in *Fig. 1, on page 8*.

Note: *The column assembly can also be used for convenient handling of columns when using the gravity protocol.*

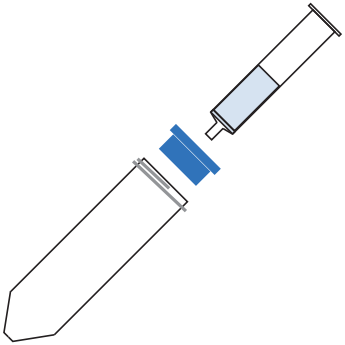

Fig 1. Assembly of column, adapter, and collection tube.

5 Gravity protocol

Column preparation

Step	Action
1	Remove the top cap and pour off the column storage solution.
2	Remove the bottom cap.

Column equilibration

Step	Action
1	Fill up the column with equilibration buffer and allow the equilibration buffer to enter the packed bed completely.

Step	Action
2	Repeat twice.
3	Discard the flowthrough. ***Note:*** *Use a total of approximately 15 mL of equilibration buffer for all three steps.*

Sample application

Step	Action
1	Add a maximum of 1.0 mL of sample to the column.
2	For sample volumes less than 1.0 mL, add equilibration buffer to adjust the volume up to 1.0 mL after the sample has entered the packed bed completely.
3	Let the sample or equilibration buffer enter the packed bed completely.
4	Discard the flowthrough.

Elution

Step	Action
1	Place a test tube for sample collection under the column.
2	Elute with 1.5 mL buffer and collect the eluate. A typical elution profile is shown in *Fig. 2, on page 12*.

6 Spin protocol

Column preparation

Step	Action
1	Remove the top cap and pour off the column storage solution.
2	Remove the top filter using forceps.
3	Remove the bottom cap.
4	Place the PD MidiTrap G-25 into a 50 mL collection tube by using the column adapter, see *Fig. 1, on page 8*.

Column equilibration

Step	Action
1	Fill up the column with equilibration buffer and allow the equilibration buffer to enter the packed bed completely.
2	Repeat once.
3	Discard the flowthrough.
4	Fill up the column a third time with equilibration buffer and spin down at 1000 × *g* for 2 minutes.
5	Discard the flowthrough. ***Note:*** *Use a total of approximately 15 mL of equilibration buffer for all three steps.*

Sample application

Step	Action
1	Add sample (0.75 to 1.0 mL) slowly in the middle of the packed bed.

Elution

Step	Action
1	Place the PD MidiTrap G-25 into a new 50 mL collection tube.
2	Elute by centrifugation 1000 × *g* for 2 minutes.
3	Collect the eluate.

7 Recovery and desalting capacity

The following experiment is included as an example of a desalting experiment using the gravity protocol. A PD MidiTrap G-25 column was equilibrated with Milli-Q water. 1 mL of bovine serum albumin 1 mg/mL in 1 M NaCl was applied onto the column. The protein recovery was 95% and the desalting capacity was above 98%, see *Fig. 2, on page 12*.

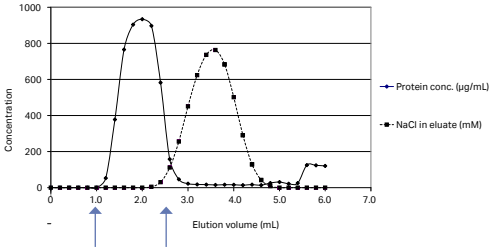

Fig 2. Removal of NaCl from albumin solution with PD MidiTrap G-25. The albumin is eluted in volume fractions between 1.0 to 2.5 mL (indicated by arrows).

8 Column characteristics

Matrix	Sephadex G-25 resin
Particle size range	85 to 260 µm
Packed bed dimensions	1.3 × 2.6 cm (3.5 mL)
Maximum sample volume	1.0 mL
Volume of eluted sample gravity	1.5 mL
Volume of eluted sample spin	1.0 mL
Desalting capacity	> 90%
Exclusion limit	M_r 5000
Chemical stability	All commonly used buffers
Working pH range	2 to 13
Storage temperature	+4°C to +30°C
Storage solution	0.15% Kathon CG/ICP biocide

9 Ordering information

Product	Pack size	Product code
PD MidiTrap G-25	50	28918008

Related products	Pack size	Product code
PD-10 Desalting Columns	30	17085101
PD SpinTrap G-25	50	28918004
PD MultiTrap G-25	4 × 96-well filter plates	28918006
PD MiniTrap G-25	50	28918007
PD MiniTrap G-10	50	28918010
PD MidiTrap G-10	50	28918011
MiniSpin Adapter	10	28923244
HiTrap™ Desalting	5 × 5 mL	17140801
HiTrap Desalting[1]	100 × 5 mL	11000329
HiPrep™ 26/10 Desalting	1 × 53 mL	17508701
HiPrep 26/10 Desalting	4 × 53 mL	17508702

[1] Pack size available by special order.

cytiva

Give feedback on this document

Visit cytiva.com/techdocfeedback or scan the QR code.

cytiva.com

For local office contact information, visit cytiva.com/contact

28922530 AE V:6 01/2023