PD-10 Desalting Column

Product Booklet

cytiva.com

52130800 BD

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1 Introduction

Product code

17-0851-01

PD-10 Desalting Column contains

- 30 prepacked disposable PD-10 Desalting Columns containing 8.3 mL of Sephadex™ G-25 resin
- 4 adapters
- Instructions for use

Purpose

PD-10 Desalting Column are prepacked and designed for rapid, convenient sample clean-up of proteins and other large biomolecules (>5000 M_r).

PD-10 Desalting Columns can be used in a wide range of applications such as desalting, buffer exchange and removal of low-molecular weight compounds.

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.

Storage

Storage temperature: 4°C to 30°C.

Storage solution: 0.15% Kathon CG/ICP biocide.

2 Principle

PD-10 Desalting Columns contain *Sephadex G-25 resin*, which allows rapid group separation of high molecular weight substances from low molecular weight substances.

PD-10 Desalting Columns are used for desalting, buffer exchange and sample clean up. Small molecules like salt, free labels and other impurities are efficiently separated from the high molecular weight substances of interest.

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 are 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 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 made using two different protocols, gravity protocol and spin protocol.

Cytiva provides an assortment of sample clean-up products. The different formats available are summarized in Table 1 below.

Table 1. Product overview.

Clean Up product	Exclusion limit, M_r	Bed volume	Sample volume gravity protocol[1]	Sample colume 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 Column	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

The separation can be made using two different protocols, gravity protocol or spin protocol, see *Table 2, on page 6* for protocol overview.

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 applied sample is diluted.

Spin protocol

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

- There is no dilution of the sample.

Table 2. Protocol overview.

Protocol	Sample volume	Elution buffer	Dilution factor	Desalting capacity
Gravity	1.0—2.5 mL	3.5 mL	1.4 times[1]	›98%
Spin	1.75—2.5 mL	None	None	›90%

[1] 1.4 times dilution valid if 2.5 mL sample volume is used.

Recovery

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

Equilibration

- It is critical to equilibrate the column since UV absorbing stabilizers are used in column packing.
- Equilibration is most conveniently made by gravity also when using the spin protocol.

Sample Application

- PD-10 Desalting Column is intended for sample volumes up to 2.5 mL.
- For sample volumes less than 2.5 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 2.5 mL.
- Allow the sample to enter the packed bed completely before any addition of buffer for elution.

LabMate buffer reservoirs

To simplify the use of PD-10 columns for gravity protocol use LabMate buffer reservoirs. For ordering information, see Chapter 9.

- Place the LabMate buffer reservoir on top of the PD-10 column.
- Add all buffer (25 mL) that should be added in step 2 in the Gravity procol (Chapter 5) in one step.

Elution

- For higher recoveries, use stacker volumes (spin protocol).

Centrifugation

- For better result if using a fixed angle rotor centrifuge: Put the columns in same direction in all centrifugation steps (spin protocol).

4 Column assembly

For use in the spin protocol the PD-10 Desalting Column must be assembled with adapter and collection tube as shown in Figure 1 below.

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

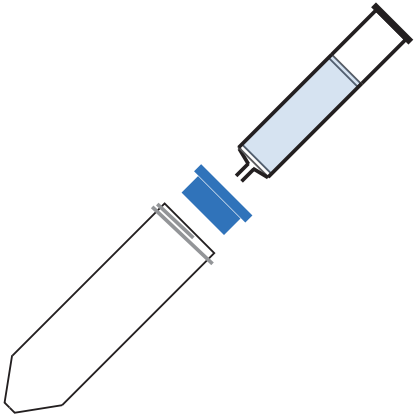

Fig 1. Assembly of column, adapter and collection tube.

5 Gravity protocol

PD-10 Desalting Column preparation

Step	Action
1	Remove the top cap and pour off the column storage solution.
2	Cut the sealed end of the column at notch.
3	Proceed with the next part of the protocol.

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 4 times.
3	Discard the flow-through. ***Note:*** *About 25 mL equilibration buffer should be used in total for all three steps.*
4	Proceed with the next part of the protocol.

Sample application

Step	Action
1	Add maximum 2.5 mL of sample to the column.

Step	Action
2	For sample volumes less than 2.5 mL, add equilibration buffer to adjust the volume up to 2.5 mL after the sample has entered the packed bed completely.Let the sample or equilibration buffer enter the packed bed completely.
3	Discard the flow-through.
4	Proceed with the next part of the protocol.

Elution

Step	Action
1	Place a test tube for sample collection under the column.
2	Elute with 3.5 mL buffer and collect the eluate. A typical elution profile is shown in *Fig. 2, on page 13*.

6 Spin protocol

PD-10 Desalting Column preparation

Step	Action
1	Remove the top cap and pour off the column storage solution.
2	Remove the top filter using forceps.
3	Cut the sealed end of the column at notch.

Step	Action
4	Put the PD-10 Desalting Column into a 50 mL collection tube by using the column adapter, see *Fig. 1, on page 8*.
5	Proceed with the next part of the protocol.

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 3 times and discard the flow-through.
3	Fill up the column a fifth time with equilibration buffer and spin down at 1000 × g for 2 minutes.
4	Discard the flow-through. ***Note:*** *About 25 mL equilibration buffer should be used in total for all three steps LabMate PD-10 Buffer Reservoir can be used for more convenient equilibration (allows loading of total 25 mL buffer at the same time).*
5	Proceed with the next part of the protocol.

Sample application

Step	Action
1	Add sample (1.75—2.5 mL) slowly in the middle of the packed bed.
2	Proceed with the next part of the protocol.

Elution

Step	Action
1	Place the PD-10 PD-10 Desalting Column 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-10 Desalting Column was equilibrated with Milli-Q™ water. 2.5 mL of bovine serum albumin 1 mg/mL) in 1M NaCl was applied onto the column. The protein recovery was 95% and the desalting capacity was above 99%, see Figure 2 below.

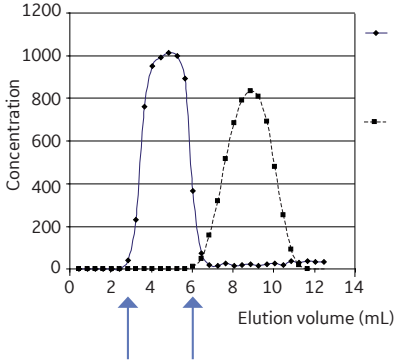

Fig 2. Removal of NaCl from albumin solution with PD-10 Desalting Column. The albumin is eluted in volume fractions between 2.5 to 6.0 mL (indicated by arrows).

8 Column characteristics

Matrix	Sephadex G-25 resin
Particle size range	85 to 260 µm
Packed bed dimensions	1.45 × 5.0 cm (8.3 mL)
Maximum sample volume	2.5 mL
Volume of eluted sample gravity	3.5 mL
Volume of eluted sample spin	2.5 mL
Desalting Capacity	>90%
Exclusion limit	M_r 5000
Chemical stability	All commonly used buffers
Working pH range	2—13
Storage temperature	+4 to +30°C
Storage solution	0.15% Kathon CG/ICP biocide

9 Ordering information

Product	Pack size	Product code
PD-10 Desalting Columns	30	17-0851-01

Related products	Pack size	Product code
PD SpinTrap G-25	50	28-9180-04
PD MultiTrap G-25	4 × 96-well filter plates	28-9180-06
PD MiniTrap G-25	50	28-9180-07
PD MidiTrap G-25	50	28-9180-08
PD MiniTrap G-10	50	28-9180-10
PD MidiTrap G-10	50	28-9180-11
PD-10 Spin Adapter	10	28-9232-45
LabMate PD-10 Buffer Reservoir	10	18-3216-03
HiTrap™ Desalting	5 × 5 mL	17-1408-01
HiTrap Desalting[1]	100 × 5 mL	11-0003-29
HiPrep™ 26/10 Desalting	1 × 53 mL	17-5087-01
HiPrep 26/10 Desalting	4 × 53 mL	17-5087-02

[1] Pack size available by special order.

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

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