

PD MidiTrap™ G-10

Instructions for Use

cytiva.com 28922533 AF

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1 Introduction

Product code

28918011

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.

2 Description

PD Midi $Trap^TM$ G-10 columns contain Sephadex TM G-10 resin, which allows rapid group separation of high molecular weight substances from low molecular weight substances.

PD MidiTrap G-10 columns are used for desalting, buffer exchange, and sample cleanup. 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 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.

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 MidiTrap 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	-

Recommended sample volumes.

3 Advice on handling

Recovery

 The recovery of applied amount sample is dependent on the type of peptide 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.

Sample application

- The MidiTrap column is intended for sample volumes up to 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 1.7 mL.
- Allow the sample to enter the packed bed completely before any addition of buffer for elution.

Optimization

For optimization of the recovery/desalting capacity, create an elution profile of the peptide/biomolecule studied, see example in *Fig. 1*, on page 8.

- Add, for example, 100 µL aliquots of equilibration buffer and collect fractions in separate tubes.
- Study the fractions by using a suitable analysis method.

4 Gravity protocol

Column preparation

Resuspend the resin by shaking the column. Allow the resin to settle. Remove the top and bottom caps. Allow the column storage solution to flow out.

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 until a total of 16 mL of equilibration buffer has been added.
3	Discard the flowthrough.

Sample application

Step	Action
1	Add a maximum of 1.0 mL of sample to the column.
2	Allow the sample to enter the packed bed completely.

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Step Action

- 3 Add equilibration buffer (stacker volume) so that the total volume of sample and buffer added equals 1.7 mL.
- 4 Let the equilibration buffer enter the packed bed completely.
- 5 Discard the flowthrough.

Elution

Step Action

- Place a test tube for sample collection under the column.
- Elute with 1.2 mL buffer and collect the eluate. A typical elution profile is shown in Fig. 1, on page 8.

5 Recovery and desalting capacity

The following experiment is included as an example of a desalting experiment. A PD MidiTrap G-10 column was equilibrated with Milli-Q water. $500\,\mu\text{L}$ of neurotensin (100 pmol/ μL) solution in 1.0 M NaCl was applied onto the column. The neurotensin recovery was 94% and the desalting capacity was above 95%, see figure below.

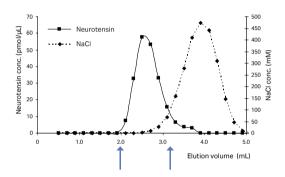


Fig 1. Removal of NaCl from a neurotensin solution with PD MidiTrap G-10. The neurotensin is eluted in volume fractions between 2.0 to 3.2 mL (indicated by arrows).

6 Column characteristics

Matrix	Sephadex G-10 resin
Particle size range	55 to 165 µm
Packed bed dimensions	1.3 × 4.0 cm (5.3 mL)
Maximum sample volume	1.0 mL
Volume of eluted sample gravity	1.2 mL

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Desalting capacity	>75%	
Exclusion limit	M_r 700	
Chemical stability	All commonly used buffers	
Working pH range	2 to 13	
Storage temperature	+4°C to +30°C	
Storage solution	20% ethanol	

7 Ordering information

Product	Pack size Pack size	Product code
PD MidiTrap G-10	50	28918011

Related products	Pack size 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
HiTrap™ Desalting	5 × 5 mL	17140801
HiTrap Desalting ¹	100 × 5 mL	11000329
HiPrep™ 26/10 Desalting	1	17508701
HiPrep 26/10 Desalting	4	17508702

¹ Pack size available by special order.





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