

# PD MidiTrap G-10

### PD MidiTrap G-10 contains

- 50 prepacked disposable PD MidiTrap™ columns containing 5.3 ml of Sephadex™ G-10
- Instructions for use

### Purpose

PD MidiTrap G-10 columns are prepacked and designed for rapid, convenient sample clean-up of small proteins, peptides, oligo-saccharides and other small biomolecules (>700 M<sub>r</sub>).

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



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## 1 Principle

PD MidiTrap G-10 columns contain *Sephadex G-10*, 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 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.

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

**Table 1.** Product overview

Clean-Up product	Exclusion limit, $M_r$	Bed volume	Sample volume gravity protocol <sup>1</sup>	Sample volume spin protocol <sup>1</sup>
PD SpinTrap™ G-25	5000	0.5 ml	-	70 to 130 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	-

<sup>1</sup> Recommended sample volumes.

## 2 Advice on handling

### Recovery

- The recovery of applied amount sample is dependent on type of peptide or other biomolecule. Typically the recovery is in the range 70-90%. 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 ensure that a equilibration volume corresponding to 3 packed bed volumes are 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

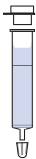
For optimization of recovery/desalting capacity, make an elution profile of the studied peptide/biomolecule, see example in Fig 1.

- Add for example 100  $\mu$ l aliquots of equilibration buffer and collect fraction in separate tubes.
- Study the fractions by a suitable analysis method.

## 3 Safety precautions

Always use normal personal protection devices like gloves and safety glasses when handling PD MidiTrap G-10 columns.

## 4 Gravity protocol



### 1 PD MidiTrap G-10 preparation

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

### 2 Column equilibration



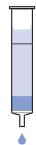
- Fill up the column with equilibration buffer and allow the equilibration buffer to enter the packed bed completely.
- Repeat until totally 16 ml equilibration buffer has been added.
- Discard the flow-through.

### 3 Sample application



- Add maximum 1.0 ml of sample to the column.
- Allow the sample to enter the packed bed completely
- Add equilibration buffer (stacker volume) so that the total volume of sample and buffer added equals 1.7 ml.
- Let the equilibration buffer enter the packed bed completely.
- Discard the flow-through.

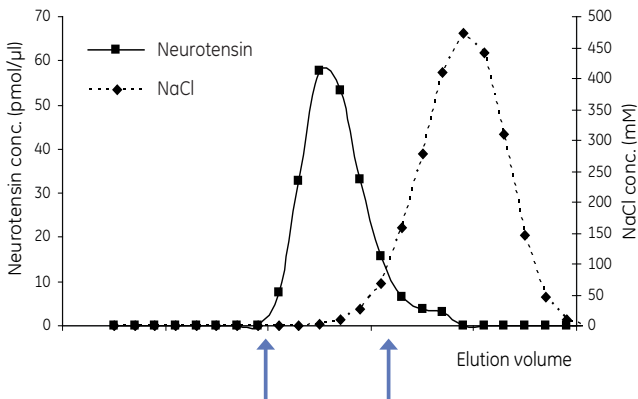
### 4 Elution



- 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.

## 5 Recovery and desalting capacity

The following experiment is included as an example of a desalting experiment. A PD MidiTrap G-10 was equilibrated with MilliQ™ water. 500  $\mu\text{l}$  of neurotensin (100 pmol/ $\mu\text{l}$ ) solution in 1.0 M NaCl was applied onto the column. The neurotensin recovery was 94% and the desalting capacity was above 95%, see Fig 1.



**Fig 1.** Removal of NaCl from a neurotensin solution with PD MidiTrap G-10. The neurotensin is eluted in volume fractions indicated by arrows.

## 6 Column characteristics

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Matrix	Sephadex G-10 Medium
Particle size range	55 to 165 $\mu\text{m}$
Packed bed dimensions	1.3 x 4.0 cm (5.3 ml)
Maximum sample volume	1.0 ml
Volume of eluted sample gravity	1.2 ml
Desalting Capacity	>75%
Exclusion limit	$M_r$ 700
Chemical stability	All commonly used buffers
Working pH range	2-13
Storage temperature	+4 to +30°C
Storage solution	20% Ethanol

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## 7 Ordering information

Product	Pack size	Code No.
PD MidiTrap G-10	50	28-9180-11

Related products	Pack size	Code No.
PD-10 Desalting Columns	30	17-0851-01
PD SpinTrap G-25	50	28-9180-04
PD MultiTrap G-25	4 x 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
HiTrap™ Desalting	5 x 5 ml	17-1408-01
HiTrap Desalting <sup>1</sup>	100 x 5 ml	11-0003-29
HiPrep™ 26/10 Desalting	1	17-5087-01
HiPrep 26/10 Desalting	4	17-5087-02

<sup>1</sup> Pack size available by special order.









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