GE Healthcare

Instructions 71-7174-00 AH **Reversed Phase Columns**

RESOURCE™ RPC 1 ml and 3 ml

Introduction

RESOURCE RPC 1 ml and 3 ml are pre-packed high performance columns for purifying peptides, proteins and other biomolecules by reversed phase chromatography (RPC). The columns are packed with SOURCE™ 15RPC chromatography medium. Use RESOURCE RPC 1 ml for rapid screening. Transfer to RESOURCE RPC 3 ml column for higher resolution and method development on a 10 cm bed height.

RESOURCE

Intended use

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

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1 Description

SOURCE 15RPC is a polymeric, reversed phase chromatography medium based on rigid, monodisperse 15 µm beads made of polystyrene/divinylbenzene. The polymer surface is underivatized; the hydrophobic interactions between the medium and the sample give a unique selectivity.

SOURCE 15RPC has a high chemical stability. The monodispersity of the beads yields stable beds and gives excellent results at high flow rates. Separations can be run in the pH range 1 to 12, which allows wide flexibility when choosing running conditions. Cleaning can be carried out in the pH range 1 to 14, which makes the use of effective cleaning procedures possible.

The relatively low back pressure versus flow rate makes it possible to use high flow rates. The maximum flow rate is 10 ml/min (1800 cm/h), while a more typical flow rate range is 1 to 5 ml/min (200 to 900 cm/h). When you run the column at 5 ml/min in distilled water at 20°C, the back pressure is normally 0.4 MPa (4 bar, 60 psi) for the 1 ml column and about 1.3 MPa (13 bar, 195 psi) for the 3 ml column.

The material of the column hardware is PEEK. The top frit is made of titanium. The bottom filter is made of polypropylene, in the 1 ml column and titanium in the 3 ml column. Table 1 summarizes the characteristics of RESOURCE RPC columns.

Table 1. Characteristics of RESOURCE RPC columns.

Medium	SOURCE 15RPC
Matrix	Monodisperse underivatized polystyrene/divinyl benzene beads
Bed volume	1 ml or 3 ml
Column dimensions, i.d. × H	
1 ml bed volume	6.4 × 30 mm
3 ml bed volume	6.4 × 100 mm
Max pressure	40 bar (4MPa, 580 psi)
Max flow rate*	10 ml/min
Typical flow rate range	1 to 5 ml/min
Particle size	15 µm
Pore volume	1.9 ml/g
Dynamic capacity**	
BSA (MW 67 000)	Approx. 10 mg/ml
Bacitracin (MW 1 to 400)	Approx. 30 mg/ml
Insulin (MW 5 700)	Approx. 50 mg/ml
pH stability	
working range	1 to 12
cleaning range	1 to 14
Operating temperature	4°C to 40°C
Delivery conditions	20% ethanol

* To measure the dynamic capacity, 5 mg bacitracin, 5 mg insulin or 2 mg BSA were applied at 300 cm/h to RESOURCE RPC, 1 ml column.

**H_2O at 25°C.

2 Preparation

Choosing an eluent system

Since polymer-based media can be used over a wider pH range and without concerns over mixed mode retention and the need to suppress the ionic interactions of silanol groups, a wider range of eluent protocols can be used.

For samples with unknown properties or known to require acidic conditions

Eluent A: 0.065% TFA in 2% acetonitrile

Eluent B: 0.050% TFA in 80% acetonitrile

For samples known to require basic conditions

Eluent A: 0.125% ammonium solution pH 10 in 2% acetonitrile

Eluent B: 80% acetonitrile in eluent A

Other systems

Eluent A: pH 2.1 0.1% formic acid, 2% acetonitrile
pH 2.0 0.1% acetic acid, 2% acetonitrile
pH 2.0 0.1% TFA, 2% acetonitrile
pH 4.5 10 mM sodium acetate, 2% acetonitrile
pH 7.0 10 mM potassium phosphate, 2% acetonotrile
pH 9.0 10 mM Tris-HCl, 2% acetonitrile in buffer
pH 12 10 mM NaOH, 2% acetonitrile in buffer

Eluent B: 70% acetonitrile in eluent A

Preparing eluent and sample

We strongly recommend that you prepare solvents and samples carefully to protect the column.

Preparing eluent and sample:

1. Filter eluents which have had solids added, using a 0.22 µm filter. This prevents particles from clogging the column.
2. Measure volumes of organic solvent and aqueous solutions separately and then mix (this eliminates volume variations which occur when mixing organic and aqueous phases directly).
3. Degas the solutions in a sonication bath (<15 min), under vacuum with magnetic stirring (<5 min) or by purging with helium (<5 min). This prevents bubble formation during elution. Be careful to keep the degassing time as low as possible in order to prevent evaporation of the organic solvent.
4. Add volatile ion pairing agents.
5. Dissolve the sample in eluent A.
6. Centrifuge samples at 10,000 g for 10 min or filter through a 0.22 or 0.45 µm sterile filter. Use a solvent-resistant filter if there is an organic modifier in eluent A. Apply to the column as soon as possible to avoid any side reactions such as oxidation.

3 Operation

Connecting the column

Before connecting the column, wash the liquid pathway of the system with the eluents you plan to use. This makes sure that none of the previous solutions remain in the tubings.

Always use a flow restrictor (compatible with an appropriate pressure range) connected after the detector of a chromtography system to prevent the accumulation of air in the detector.

Equilibrating the column

The column is delivered in 20% ethanol. When you equilibrate the column for first time use, after long term storage, or when changing eluents, proceed according to steps 1 to 3 below.

1. Wash the column with approximately 3 column volumes of eluent B at a low to moderate flow rate.
2. Run a 2 to 3 column volume linear gradient from 100% eluent B to 100% eluent A at the same flow rate as in step 1.
3. Equilibrate the column with 10 column volumes of eluent A. Continue equilibration until all monitor signals are stable.

Separation by gradient elution

Flow: 1 to 5 ml/min

Collect fractions throughout the separation.

1. Equilibrate the column with at least 10 column volumes of eluent A until the UV signal is stable.
2. Dissolve the sample in a small volume of eluent A. Filter or centrifuge to remove particulate matter if necessary. Apply to the column.
3. When the UV signal is stable, that is, when all unbound material has washed through the column, elute using a gradient of 10 to 20 column volumes from 0% to 100% eluent B.

4 Wash the column with at least 5 column volumes of 100% eluent B (or until UV signal is stable) to elute any remaining material.

5 Wash with a gradient of 2 to 3 column volumes from 0% to 100% eluent B.

6 Re-equilibrate with 10 column volumes of eluent A or until UV signal is stable.

4 Maintenance

Storage

When you have finished using the column, wash it with at least 10 column volumes distilled water, equilibrate with at least 10 column volumes 20% ethanol or 70% acetonitrile. Store the column at 4°C to 30°C. Ensure that the column is sealed well to avoid drying out. Do not freeze.

Cleaning

Correct preparation of samples and eluents, including filtration, the removal of any particulate matter and a final wash step in 100% eluent B, should keep most columns in good condition. However, reduced performance, reduced flow, increasing back pressure or complete blockage are all indications that the medium needs to be cleaned using more stringent procedures in order to remove tightly bound, precipitated or denatured substances.

It is not recommended to reverse the direction of flow due to the column design. The number of column volumes and contact time required for each cleaning step may vary according to the degree of contamination.

Contact time, organic solvent and pH are significant parameters for successful cleaning, and different protocols may have to be developed and used in combination according to the nature of the contaminants.

Examples of cleaning protocols are as follows:

Eluent A: 0.1% TFA

Eluent B: 0.1% TFA in 80% acetonitrile

Flow: 1.0 ml/min

1. Equilibrate the column with at least 10 column volumes of eluent A until the UV signal is stable.
2. Wash using a gradient of 20 to 30 column volumes from 0% to 100% eluent B.
3. Wash the column with at least 10 column volumes of 100% eluent B.
4. Wash using a gradient of 20 to 30 column volumes from 0% to 100% eluent B.
5. Wash the column with at least 10 column volumes of eluent A.
6. Equilibrate the column in at least 10 column volumes in the eluent A that will be used for the separation if different the eluent used in step 5. Transfer between the two eluents should be performed using a 2 to 3 column volume gradient if the two eluents are significantly different.

Change to 0.1%TFA in 2-propanol for eluent B if column performance is not restored. Note that 2-propanol will increase back-pressure, and flow rates may need to be reduced.

For removal of contaminants known to be acid- or alkali-soluble the following eluents can be used, following the same procedure as outlined above:

Removal of acid-soluble contaminants

Eluent A: 90% acetic acid

Eluent B: 80% acetonitrile or 50% 2-propanol

Removal of alkali-soluble contaminants

Eluent A: 0.5 M NaOH

Eluent B: 50% acetonitrile or 50% 2-propanol

If neither of the protocols for acid- or alkali-soluble contaminants is successful, wash the column in 5 to 10 column volumes of 6 M guanidine hydrochloride.

5 Ordering information

Product	Quantity	Code No.
RESOURCE RPC 1 ml	1	17-1181-01
RESOURCE RPC 3 ml	1	17-1182-01

Accessories	Quantity	Code No.
Union M6 female/1/16" male (for connection to FPLCTM systems)	5	18-3858-01
Fingertight connector 1/16" (for connection to ÄKTA™ design systems)	10	18-1112-55

Related Products	Quantity	Code No.
SOURCE 15RPC	10	17-0727-20
Hydrophobic Interaction & Reversed Phase Chromatography, Principles and Methods		11-0012-69

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