instructions

RESOURCE ETH, 1ml
RESOURCE ISO, 1 ml
RESOURCE PHE, 1 ml
RESOURCE HIC Test Kit

ⓘ 56-1188-26 AB

Amersham Biosciences

Introduction

RESOURCE™ ETH (ether), ISO (isopropyl) and PHE (phenyl) are pre-packed high performance columns for separating biomolecules by hydrophobic interaction chromatography (HIC) HIC gives fast, high resolution purification of proteins and peptides. The columns can be conected to ÄKTA™ systems or other chromatography systems using connectors supplied with each column. In general, RESOURCE PHE will have the strongest hydrophobicity followed by RESOURCE ISO and RESOURCE ETH succesively.

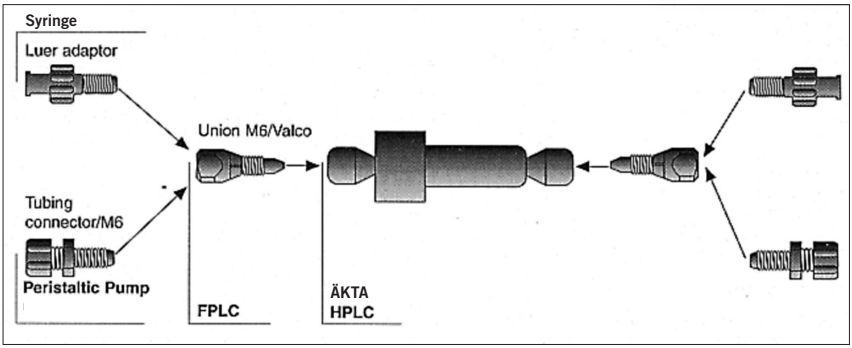

Fig. 1. RESOURCE column and connectors

Refer to Figure 1 and check that your package contains the followin items:

Item	Code No.	No. per pack
RESOURCE ETH	17-1184-01	1
or		
RESOURCE ISO	17-1185-01	1
or		
RESOURCE	17-1186-01	1
or		
RESOURCE HIC Test Kit: RESOURCE ETH, ISO and PHE	17-1187-1	1 of each column
Connectors:		
Union M6/Valco		2
Luer adaptor		2
Tubing connectors/M6		2
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Product description

RESOURCE ETH, ISO and PHE are hydrophopic interaction columns prepacked with SOURCE™ 15 ETE , SOURCE 15 ISO or source PHE with different characteristics based on rigid, monodisperse 15 µm beads made of polystyrene/divinyl benzene. The base matrix of these HIC media has been hydrophilised prior to coupling with the hydrophobic ligands. Their low back pressure at high flow rates makes high resolution separations attainable even when using a low-pressure peristalic pump. In addition, hydrophilisation of the beads minimizes non-specific adorption and a follows high recovery of purified sample.

RESOURCE ETH, ISO and PHE are stable in pH 1-14 for cleaning and in pH 2-12 for operation and storage. They are stable in denaturing agents, detergents, chaotropic agents and most commonly used organic solvents

The material of the column body is PEEK (polyetheretherketone). The top frit is made of PAT (PEEK alloyed to tetraflouroethylene, TFE**). The bottom filter is made of polypropylene. The maximum pressure stability of the column is 1.5 MPa (15 bar, 220 psi). Do not exceed this pressure. The gel bed withstands a maximum pressure drop of 1.5 MPa (15bar, 217 psi)

Table 1 summerizes the charactaristics of RESOURCE ETH, ISO and PHE 1 ml columns.

Table 1. Characteristics of RESOURCE ETH, ISO and PHE 1 ml columns.

Column dimensions	6.4 mm i.d. x 30 mm
Gel volume	1 ml
Base matrix	Monodisperse polystyrene/divinyl benzene beads
Particle size	15 µm
Surface area	70m^2/g
Pore size	200-10 000 Å
Pore volume	1.0 ml/g
Practical loading range (proteins)[1]	At least 25 mg albumin/ml
pH stability	
working range	2-12
cleaning range	1-14
Chemical stability	Stable in 1m HCl, 2 M NaOH, 100% isopropanol, 10% isopropanol/0.5 M NaOH, 20% ethanol (all tested at 40°C for 7 days) and in all comonly used aqueous buffers.
Maximum flow rate[2]	9.6ml/min
Typical working flow rate range	0.8.4.8 ml/min
Pressure limit, gel bed	1.5 MPa (15 bar, 220 psi)
Operating temperatur	4-40 °C

1 Detremined by Frontal analysis at a flow rate of 1.6 ml/min using a 5.0 mg/ml solution of albumin in 100mM potassium phosphate pH 7.0 containing 2.0 M ammonium sulphate. 2 In water at 25°C.

Type of ligand

The choice between different types of ligand is empirical and is established by a screening experiment for each individual problem. The goal witha the media selection will be to find a medium that gives strong binding at a reasonably low salt concentration. The salt concentration should be below the concentration that causes precipitation and aggregation. for example, 1.5 M ammonium sulphate is a good starting point for a screening experiment. If the substance of interest does not bind under such conditions, try a more hydrophobic medium.

Preparation

Choosing the buffer system

Once the sample has been adsorbed at a high ionic strenght, ther are different ways to desorb it. one way is to utilize a gradient of decreasing ionic strenght to selectively desorb components according to their hydrophobicity. Other ways to achieve desorption include changing to an ion with lower salting-out effect (see Table 2), lowering the polarity of the eluent e.g. by adding ethylene glycol. including a detergent in the eluent and changing the pH of the eluent.

Table 2. Hofmesister series

<—Increasing preciptation ("salting-out") effect
Anions:PO_4^{3-}, SO_4^{2-}, CH_3COO^-, Cl^-, Br^-, NO_3^-, ClO_4^-, I^-, SCN^-
Cations: NH_4^+, Rb^+, K^+, Na^+, Cs^+, Li^+, Mg^+, Ca^{2+}, Ba^{2+}
Increasing chaotropic ("salting in") effect—>

Increasing the salting-out effect strengthens the hydrophobic interactions, whereas increasing the chaotropic effect weakens them.

Temperature also influences the hydrophobic iteractions. If samples adhere too strongly to the matrix, a lower temperature will decrease the interactions and sometimes improv peak symmetry.

To start with, we recommend a linear gardient from 0 to 100% elution buffer with:

Start buffer:	50mM phosphate buffer, pH 7.0 + 1.5 M $(NH_4)_2SO_4$
Elution buffer:	50mM phosphate buffer, pH 7.0

We recommend a flow rate of 1.0 ml/min and gradients of 10-20 ml. Elute the sample with gradient from 0-100% elution buffer. Before the next injektion, run your column at 100% elution buffer until the base-line is stable, the re-equilibrate at the chosen start conditions.

You can find more information about hydrophobic interaction chromotagraphy in the Amersham Biosciences handbook "Hydrophobic Interaction Chromotagraphy, Princeples and methods" Code No. 18-1020-90.

Preparing buffers and sample

To protect the column and prolong its life, we strongly recommend you prepare the buffers and sam-

ples with care. Use water of Milli-Q grade or corresponding quality. Use HPLC grade solvents, salts and buffers. (With some grades of $(NH_4)_2SO_4$ base-line drift may be observed. To eliminate this effect, $(NH_4)_2SO_4$ may be purified by treatment activated charcoal). Degas and filter all buffer solutions through a 0.22 µm filter. The sample should either be filtered through a 0.22 µm or centrifuged (10 000x g for 10 min.). When possible, disolve or dilute the sample in start buffer. Be sure to select a solvent resistant filter if samples are disolved in oraganig solvents. The sample should be fat-free. Turbid solutions can decrease the column lifetime. Buffer exchange and desalting are easily accomplished by gel filtration. We recommend HiTrap™ PD-10 columns pre-packed with Sepadex™ G-25 for this task.

Operation

General

Whenever possible, we advise dedicating an individual RESOURCE column to each particular application. We recommend you to record the basic details of each run in an operating log. Recording the number of runs helps build up a good idea of the expected liftime of the column for each application. This record, together with careful buffer and sample preperation, helps you plan regular column regular cleaning and confidently predict column performance over its working life.

Note: Altough high performance seperations with RESOURCE ETH, ISO and PHE do not put special demands on the pump, resolution obtained on the column can be lost through mixing in dead spaces afterwards. Low dead volumes and a good mixer, detector and fraction collector are essential for good results.

Connecting the column

The set of connectors included in each package lets you connect the column to different chromatography equipment (see Fig. 1)

Equilibrating and regenerating the column

The column is supplied in 20% ethanol. Follow steps 1-2 below when you equilibrate the column for first time use, after long time storage, or when changing buffers.

1. Flush the column with a minimum of five column volumes of elution buffer. This washes out most of the storage solution. Start with a flow of 0.2ml/min.
2. Run five column volumes of start buffer through the column.

Applying and eluting the sample

The practical loading capacity for RESOURCE ETH, ISO and PHE is up to 25 mg of proteins and peptides. However, this may vary depending on the peptides/proteins to be separated, on the running conditions and the detection sensivity and/or volume used.

The sample volume is of minor importance when gradient elution is used, but it can affect the resolution of early eluting components.

Optimizing gradient shape

Optimize the shape of your gradient to give the best separation. For separating peptides/proteins, we recommend starting with a linear gradient of 0-100% elution buffer in a volume of 10-20 ml. Lower flow rates and more shallow gradients usally improve resolution. If the peptide/proteins of interest is not eluted in the gradient, change the elution buffer to one with a higher salt concentration. When optimising a seperation, also consider varying critical parameters such as type and concentration of salt, pH, sample load, gradient and flow rate in a controlled way.

If the column seems to be contaminated, clean it according to the procedure recommended below.

Maintenance

Column cleaning

Think about the possible causes of contamination when choosing a cleaning method. Try to design a methos specific for the substances you suspect may remain on the column. (As recommended previously, dedicating an individual column to a specific application and keeping a record of its use will help you design effective cleaning procedures. For strongly bound hydrophobic proteins, lipoproteins and lipids, we suggests the following procedure:

Use 4-10 ml 70% ethanol or 30% isopropanol in water followed by 3-4 ml of water. Apply gradients to avoid air bubble formation when changing to/from high concentrated organic solvents.

Alternatively, use 1-2 ml of 0.5% non-ionic detergent (e.g. in 1 M acetic acid) followed by 5 ml of 70% ethanol and 3-4 ml of water.

If you are unsure what may be causing the problem, we suggest the following method, wich works for cleaning and sanitization:

Wash the column with 4 ml 0.5-1 M NaOH at a flow rate of 0.2 ml/min, followed by 2-3 ml of water.

We sugest you to install the column uppside down so that the direction of flow is reversed. After cleaning, equilibrate in a buffer to retain the pH. Remeber that you should not store the column in strong aidic or alkaline solutions (below pH 2 or above pH 12).

If the cleaning procedure does not restore satisfactory performance, replace the column with a new one.

Storage

After the column has been used, wash it with a minimum of five column volumes of 20% ethanol to prevent microbial growth. We recommend you to store the column at 4 to 30°C.

Ordering information

Column	Code No.
RESOURCE™ ETH, 1 ml	17-1184-01
RESOURCE™ ISO, 1 ml	17-1185-01
RESOURCE™ PHE, 1 ml	17-1186-01
RESOURCE™ HIC Test Kit	17-1187-01
RESOURCE columns for ion exchange	
RESOURCE™ Q, 1 ml	17-1177-01
RESOURCE™ S, 1 ml	17-1178-01
RESOURCE™ Q, 6 ml	17-1179-01
RESOURCE™ S, 6 ml	17-1180-01
RESOURCE columns for RPC	
RESOURCE™ RPC, 1 ml	17-1181-01
RESOURCE™ RPC, 3 ml	17-1182-01
Buffer exchange and desalting	
HiTrap Desalting, 5x5 ml	17-1408-01
PD-10 Desalting columns	17-0851-01

The media are also available in packs. Ask for details of quantities.

Important Information

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