GE Healthcare

Instructions 56-1188-26 AE Hydrophobic Interaction Columns

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

Introduction

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

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

RESOURCE ETH, ISO and PHE are hydrophopic interaction columns prepacked with SOURCE™ 15ETH, SOURCE 15ISO or SOURCE 15PHE 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 adsorption and follows high recovery of purified sample.

RESOURCE ETH, ISO and PHE are stable in pH 1 to 14 for cleaning and in pH 2 to 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 titanium. The bottom filter is made of polypropylene. The maximum pressure stability of the column is 1.5 MPa (15 bar, 220 psi).

Table 1 summarizes the characteristics 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. × 30 mm
Bed volume	1 ml
Base matrix	Monodisperse polystyrene/divinyl benzene beads
Particle size	15 µm
Practical loading range (proteins)[1]	At least 25 mg albumin/ml
pH stability working range cleaning range	 2 to 12 1 to 14
Chemical stability	Stable in 1m HCl, 2 M NaOH, 100% isopropanol, 10% isopropanol/ 0.5 M NaOH, 20% ethanol (both tested at 40°C for 7 days) and in all commonly used aqueous buffers.
Maximum flow rate[2]	9.6 ml/min
Typical working flow rate range	0.8 to 4.8 ml/min
Max. pressure	1.5 MPa (15 bar, 220 psi)
Operating temperature	4°C to 40°C

[1] Determined by Frontal analysis at a flow rate of 1.6 ml/min using a 5.0 mg/ml solution of albumin in 100 mM 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 with 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.

2 Preparation

Choosing the buffer system

Once the sample has been adsorbed at a high ionic strength, there are different ways to desorb it. One way is to utilize a gradient of decreasing ionic strength to selectively desorb components according to their hydrophobicity. Other ways to achieve desorption includes 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. Hofmeister series.

<—Increasing precipitation ("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 interactions. If samples adhere too strongly to the matrix, a lower temperature will decrease the interactions and sometimes improve peak symmetry.

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

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

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

You can find more information about hydrophobic interaction chromotagraphy in the GE Healthcare handbook "Hydrophobic Interaction and Reverse Phase Chromotagraphy".

Preparing buffers and sample

To protect the column and prolong its life, we strongly recommend you prepare the buffers and samples 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 (10 000 × g for 10 min). When possible, dissolve or dilute the sample in start buffer. Be sure to select a solvent resistant filter if samples are dissolved in organic solvents. Turbid solutions can decrease the column lifetime. Buffer exchange and desalting are easily accomplished by using HiTrap™ Desalting, HiPrep™ 26/10 Desalting or PD-10 Desalting columns depending on sample volume.

3 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 lifetime of the column for each application. This record, together with careful buffer and sample preparation, helps you plan regular column cleaning and confidently predict column performance over its working life.

Note: *Although high performance separations 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.*

Equilibrating and regenerating the column

The column is supplied in 20% ethanol. Follow steps 1 to 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 0.2 ml/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 sensitivity 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% to 100% elution buffer in a volume of 10 to 20 ml. Lower flow rates and more shallow gradients usually improve resolution. If the peptide/proteins of interest are not eluted in the gradient, change the elution buffer to one with a higher salt concentration. When optimizing a separation, 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.

4 Maintenance

Column cleaning

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

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

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

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

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

Due to the column design, it is not recommended to reverse the direction of flow. After cleaning, equilibrate in a buffer to retain the pH. Remember that you should not store the column in strong acidic 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.

5 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°C to 30°C.

6 Ordering information

Product	Quantity	Code No.
RESOURCE ETH, 1 ml	1	17-1184-01
RESOURCE ISO, 1 ml	1	17-1185-01
RESOURCE PHE, 1 ml	1	17-1186-01
RESOURCE HIC Test Kit	3 x 1 ml	17-1187-01

Accessories	Quantity	Code No.
Union M6 female/1/16" male (for connection to FPLC™ 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 15ETH	50 ml	17-5186-01
SOURCE 15ISO	50 ml	17-0148-01
SOURCE 15PHE	50 m	17-0147-01
HiTrap Desalting	5 x 5 ml	17-1408-01
HiPrep 26/10 Desalting	1	17-5087-01
HiPrep 26/10 Desalting	4	17-5087-02
PD-10 Desalting columns	30	17-0851-01
Hydrophobic Interaction and Reverse Phase Chromotography Handbook		11-0012-69

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