SOURCE™ 15HIC

SOURCE 15ETH, SOURCE 15ISO, SOURCE 15PHE, SOURCE 15PHE 4.6/100 PE (Tricorn™), RESOURCE™ ETH, RESOURCE ISO, RESOURCE PHE, RESOURCE HIC Test Kit

SOURCE 15ETH (ether), SOURCE 15ISO (isopropyl) and SOURCE 15PHE (phenyl) are high-performance media for hydrophobic interaction chromatography and are designed for rapid, high resolution purification of proteins and peptides. They are ideal for the final stage of a purification process where trace contaminants and close variants must be removed.

SOURCE 15ETH, SOURCE 15ISO and SOURCE 15PHE are available in media packs and in prepacked RESOURCE columns. SOURCE 15PHE is available in prepacked Tricorn high performance columns

RESOURCE columns are designed for fast separations and selectivity screening experiments. They run on any chromatography system: ÄKTA™ design or other high performance systems, low to medium pressure systems, or even with a simple peristaltic pump. This versatility makes the columns widely applicable. The three columns are available individually as well as combined in the RESOURCE HIC Test Kit.

Tricorn high performance columns are for high resolution purification at laboratory scale and for optimization studies when scaling up. It runs on ÄKTA design and other high performance systems.

SOURCE HIC media are characterized by:

- High resolution separations at high loads and high flow rates
- Wide range of selectivities
- Reproducible quality
- Excellent scalability
- High chemical and physical stability
- Low backpressure

Fig 1. SOURCE 15ETH, Source 15ISO, SOURCE 15PHE in media packs and prepacked Tricorn and RESOURCE columns.

Characteristics

SOURCE 15ETH, SOURCE 15ISO and SOURCE 15PHE are based on 15 µm, monosized, rigid, polystyrene/divinyl benzene matrices with an optimized pore size distribution. The base matrix has been hydrophilised prior to coupling with ether, isopropyl and phenyl hydrophobic ligands. During development we have focused on quality, reproducibility and scalability, features that are particularly important for industrial applications where strict regulatory demands apply. Table 1 lists the main characteristics of these three HIC media.

Table 1. Characteristics of SOURCE 15ETH, SOURCE 15ISO and SOURCE 15PHE media

Matrix	Polystyrene/divinyl benzene
Type of ligand	*Ether (ETH):* R–O–CH_2–CHOH–CH_2–OH
	Isopropyl (ISO): R–O–CH_2–CHOH–CH_2–O–CH_2–CHOH–CH_2–O–CH–$(CH_2)_3$
	Phenyl (PHE): R–O–CH_2–CHOH––CH_2–O–CH_2–CHOH–CH_2–O–C_6H_5
Bead form	Rigid, spherical, porous, monodispense
Particle size	15 µm
Dynamic capacity*	At least 25 mg albumin/ml
pH stability	
working range	2–12
cleaning range	1–14
Operating temp.	4°C–40°C
Delivery conditions	20% ethanol

* Determined by frontal analysis at a flow rate of 300 cm/h using a 5.0 mg/ml solution of albumin in 100 mM potassium phosphate pH 7.0 containing 2.0 M ammonium sulphate. The actual loading capacity in a working application will depend on the nature and concentration of contaminants in the sample, and on the degree of resolution required.

High resolution separations at high loads and high flow rates

SOURCE 15 matrices consist of beads with a uniform 15 µm diameter and a spherical shape. They are free from broken beads, fragments and fines (Fig 2).

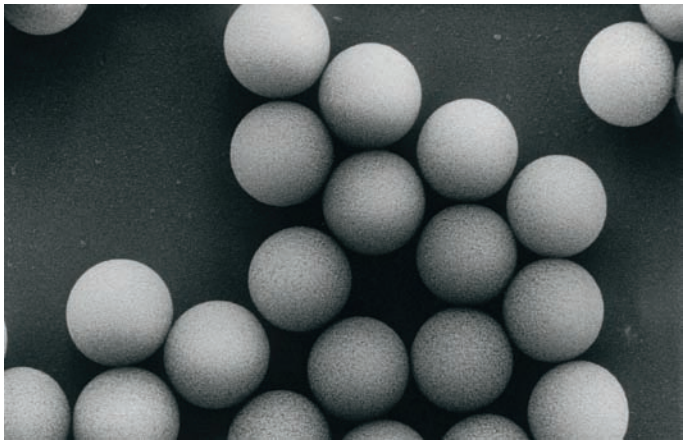

Fig 2. Electron micrograph of SOURCE 15 beads. Note the uniform size and the absence of fines, fragments and broken beads.

SOURCE 15HIC media give very high resolution. Performance is well maintained at high flow rates. This is illustrated in Figure 3, which shows the influence of increasing flow rate on the resolution of a protein mixture separated on RESOURCE PHE.

The pore size distribution of the matrix has been chosen to give high capacities for proteins and peptides. Figure 4 shows the influence of increasing load on the resolution of a protein mixture separated on SOURCE 15ISO.

Column: RESOURCE PHE, 1 ml
Sample: Mixture of myoglobin, ribonuclease, lysozyme and chymotrypsinogen
Sample load: 0.38 mg
Start buffer (A): 2.0 M ammonium sulphate, 100 mM potassium phosphate, pH 7.0
Elution buffer (B): 100 mM potassium phosphate, pH 7.0
Flow rate: a) 1.6 ml/min (300 cm/h)
b) 4.8 ml/min (900 cm/h),
c) 9.6 ml/min (1800 cm/h)
Gradient: 20%–100% B, 20 column volumes

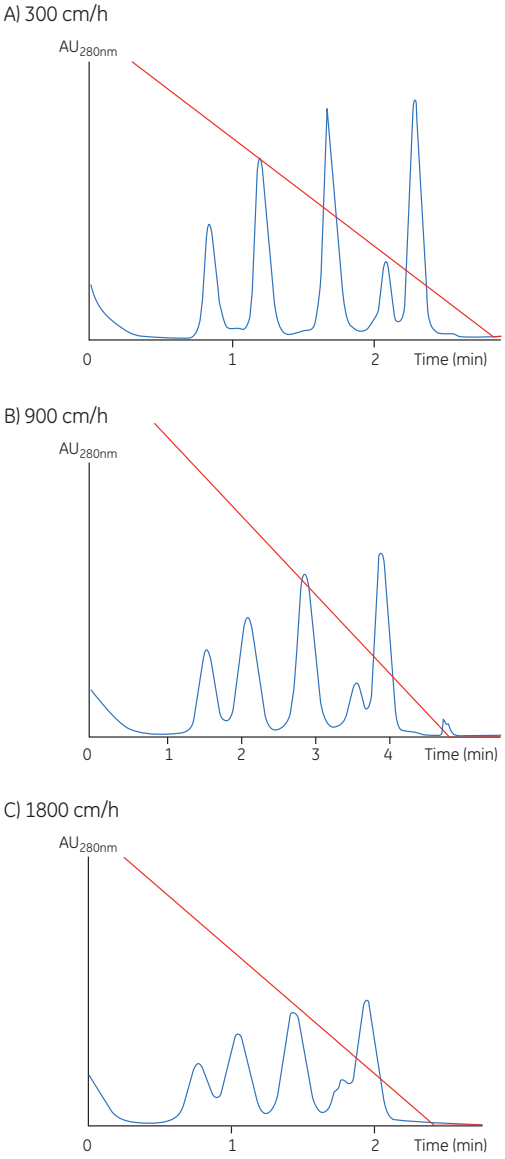

Fig 3. The influence of increasing flow rate on resolution when separating a model protein mixture on RESOURCE PHE.

Column: SOURCE 15ISO, 6.4 mm i.d., × 30 mm (1 ml)
Sample: Mixture of lactalbumin and chymotrypsinogen
Sample load: a) 0.3 mg
b) 6.4 mg
Start buffer (A): 2.0 M ammonium sulphate,
100 mM potassium phosphate, pH 7.0
Elution buffer (B): 100 mM potassium phosphate, pH 7.0
Flow rate: 1.6 ml/min (300 cm/h)
Gradient: 20%–100% B, 20 column volumes

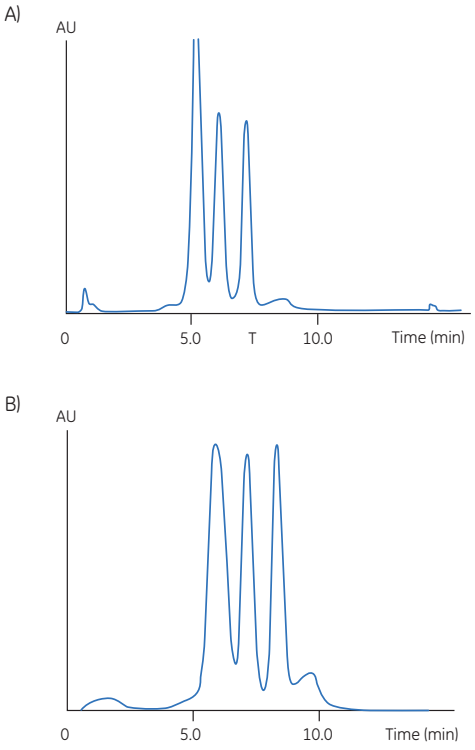

Fig 4. The influence of increasing load on resolution when separating a model protein mixture on SOURCE 15ISO.

Column: SOURCE 15ISO, 3 separate batches,
6.4 mm i.d. × 30 mm column (1 ml)
Sample: Myoglobin, ribonuclease, lactalbumin and chymotrypsinogen
Sample load: 0.3 mg
Start buffer (A): 2.0 M ammonium sulphate,
100 mM potassium phosphate, pH 7.0
Elution buffer (B): 100 mM potassium phosphate, pH 7.0
Flow rate: 1.0 ml/min (200 cm/h)
Gradient: 0%–100% B, 20 column volumes

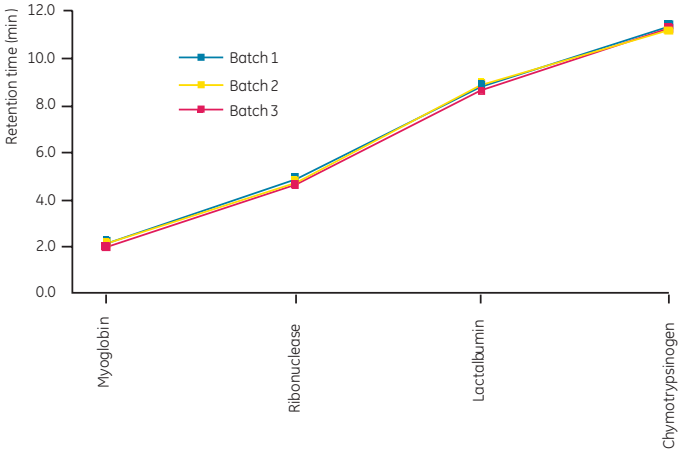

Fig 5. Retention data from QC of 3 production batches of SOURCE 15ISO.

Wide range of selectivities

The best way to choose the most suitable hydrophobic ligand for a particular separation problem is screening a range of media. In general, SOURCE 15PHE will have the strongest retention, followed by SOURCE 15ISO and SOURCE 15ETH. The three media cover a wide range of selectivities, providing the chromatographer the possibility finding the optimal choice for each particular separation.

High batch-to-batch reproducibility

The combination of the unique manufacturing process and high quality assurance standards result in consistent quality from batch to batch. Figure 5 shows quality control data of the chromatographic function of three production batches of SOURCE 15ISO.

Excellent scalability

The monosized SOURCE 15 media maintain performance during scale-up. They are easy to pack at both laboratory and large scales and give relatively low backpressures.

High chemical stability

The derivatized polymeric matrices have high chemical stability and can be used over a wide pH range (see Table 1), allowing good flexibility in choosing separation conditions and efficient cleaning and sanitization methods.

Low backpressures

The uniform size and spherical shape of the beads give stable packed beds and low backpressures in contrast to beds with a wide range of particle sizes. Figure 6 shows the pressure/flow characteristics of RESOURCE HIC columns.

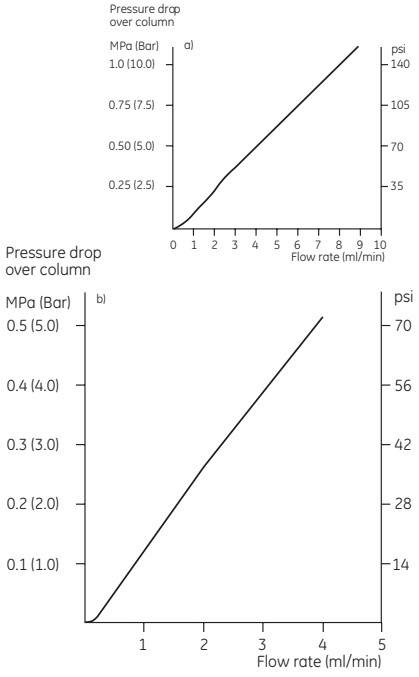

Fig 6. The influence of increasing load on resolution when separating a model protein mixture on SOURCE 15ISO.

RESOURCE HIC columns

RESOURCE HIC columns are pre-packed with SOURCE 15HIC media. They give rapid, high capacity, high resolution separations of biomolecules on ÄKTA design and other high-performance systems. The columns generate low backpressures, typically around 1 bar (0.1 MPa, 14 psi) at flow rates of 1 ml/min, which makes high resolution separations achievable even with a system based on a peristaltic pump.

The column body is made of PEEK (polyetheretherketone), which tolerates high pressure and is chemically resistant. Table 2 lists the main chromatographic properties of the three RESOURCE HIC columns.

RESOURCE HIC Test Kit

This test kit comprises the three pre-packed RESOURCE 1 ml columns (ETH, ISO and PHE). It allows for rapid screening of all three media to find the one best suited for specific applications or process development.

Tricorn High Performance Column

SOURCE 15PHE 4.6/100 PE is a Tricorn high performance column pre-packed with SOURCE 15PHE. Its high capacity makes it an excellent choice for preparative hydrophobic interaction purifications at laboratory scale. In addition, its longer bed height provides increased resolution. The column is also useful for optimization studies during scale-up.

SOURCE 15PHE 4.6/100 PE runs on ÄKTA design and other chromatography high-performance systems. The column body of SOURCE 15PHE 4.6/100 PE is made of PEEK. The main chromatographic properties of this column are listed in Table 2.

Table 2. Main chromatographic properties of columns pre-packed with SOURCE 15ETH, SOURCE 15ISO and SOURCE 15PHE

	RESOURCE ETH RESOURCE ISO RESOURCE PHE	SOURCE 15PHE 4.6/100 PE
Column dimensions		
i.d. × bed height	6.4 × 30 mm	4.6 × 100 mm
Bed volume	1 ml	1.7 ml
Recommended flow rate	0.8–4.8 ml/min	0.5–2.5 ml/min
Max. flow rate (ml/min)	10	5.0
Max. backpressure (MPa, bar, psi)	1.5, 15, 220	4, 40, 580

Operation

Method design and optimization

Choosing the most suitable HIC gel is often a matter of screening a set of different media for each individual separation problem. The medium with the best selectivity, resolution, and loading capacity at a reasonably low salt concentration will be the one most suitable for that application. In general, SOURCE PHE will give the strongest retention followed by SOURCE ISO and SOURCE ETH.

Binding to HIC adsorbents is promoted by moderately high concentrations of anti-chaotropic salts in the equilibration buffer and sample solution. Typical binding conditions are 0.05 M sodium phosphate, pH 7.0, with 1.5 M ammonium sulphate.

Bound substances are eluted by reducing the concentration of salting out ions in the buffer with a decreasing salt gradient (linear or step). A suggested elution buffer is 0.05 M sodium phosphate, pH 7.0 and a gradient of 0%–100% B in 10–20 gel bed volumes.

Use of 30% isopropanol and 0.5–1 M NaOH will normally prove effective for cleaning and sanitization.

SOURCE media have excellent flow properties and can be used in laboratory scale columns at flow rates up to 1800 cm/h. This is useful for laboratory-scale preparative applications and for scouting different separation conditions at small scale prior to scaling up. All SOURCE media are also available in bulk.

Details of general HIC theory, experimental design and technique can be found in the GE Healthcare handbook: Hydrophobic Interaction and Reversed Phase Chromatography – Principles and Methods (Code No. 11-0012-69).

The SOURCE product line also includes prepacked columns for ion exchange and reversed phase chromatography. These media are also available in bulk quantities.

Optimal running conditions

Optimal running conditions differ from application to application and are best established by varying the critical parameters in a controlled way, optimizing the type and concentration of salt, pH, sample load, gradient and flow-rate. Figure 7 shows an optimization series of eight runs to find the optimal running condition.

Column:	SOURCE 15PHE 4.6/100 PE (Tricorn)
Sample:	Recombinant Protein Tyrosine Phosphatase partially purified on HiTrap Q XL, 5 ml
Sample load:	10 ml
Start buffer (A):	25 mM Tris pH 7.5, 1 mM EDTA, 2 mM DTT (run 1)
Elution buffer (B):	A+1.5 M $(NH_4)_2SO_4$ (run 1)
Start buffer (A):	50 mM Tris pH 7.5, 1 mM EDTA, 2 mM DTT, 10% glycerol (run 4)
Elution buffer (B):	A+1.5 m $(NH_4)_2SO_4$ (run 4)
Start buffer (A):	20 mM MES pH 6.5, 1 mM EDTA, 2 mM DTT, 10% glycerol (run 7)
Elution buffer (B):	A+1.5 M $(NH_4)_2SO_4$ (run 7)
Start buffer (A):	20 mM MES pH 6.5, 1 mM EDTA, 2 mM DTT, 10% glycerol (run 8)
Elution buffer (B):	A+2 M $(NH_4)_2SO_4$ (run 8)
Flow rate:	1.5 ml/min (run 1), 1.0 ml/min (run 4, 7, and 8)
Gradient:	0%–100% B in 20 CV (run 1, 4, and 7), 27 CV (run 8)
System:	ÄKTAexplorer™ 10 at room temperature
Detection:	$A_{280\ nm}$

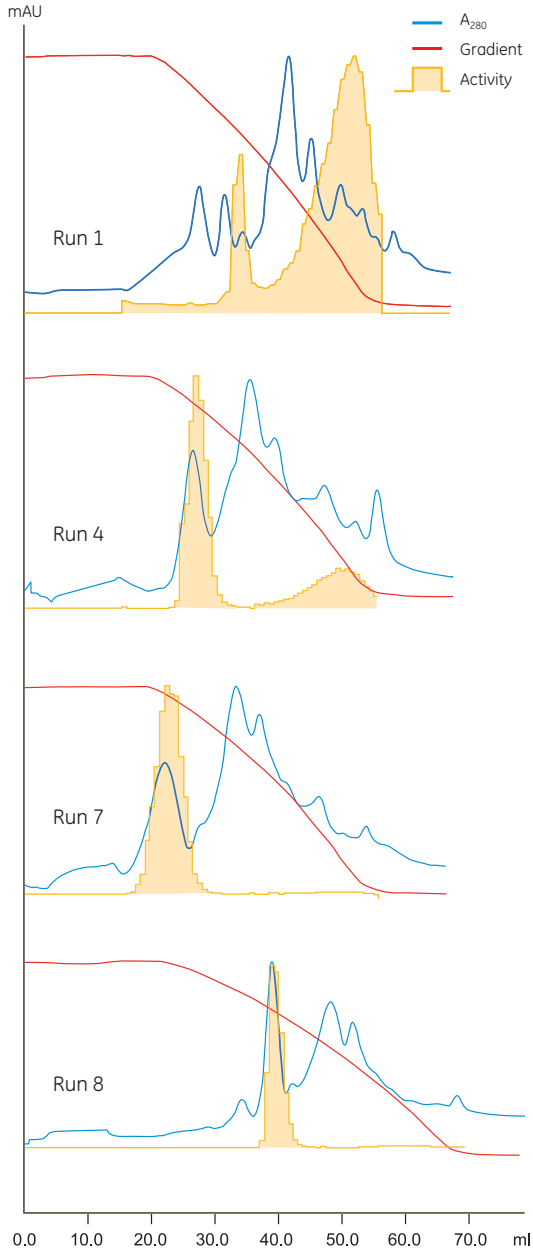

Fig 7. The figure show 4 runs in an optimization series of 8 runs to find the optimal running conditions for the purification of protein tyrosine phospatate on SOURCE 15PHE 4.6/100 PE (Tricorn). In the optimization series was the concentration of ammonium sulphate, gradient length, pH, flow rate and additives varied. The activity was measured by p-nitrophenyl phosphate (pNPP) activity assay at 405 nm. The increase of concentration of ammonium sulphate from 1.5 M to 2.0 M and the increase of the length of the gradient resulted in a better separation.

Ordering information

Column	Code No.
SOURCE 15PHE 4.6/100 PE	17-5186-01
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

All columns are supplied with instructions for use. RESOURCE columns are supplied with connectors for high and low pressure chromatography equipment.

SOURCE HIC media

Column	Pack size	Code No.
SOURCE 15ETH	50 ml	17-0146-01
	200 ml	17-0146-02
	1 l	17-0146-04
SOURCE 15ISO	50 ml	17-0148-01
	200 ml	17-0148-02
	1 l	17-0148-04
SOURCE 15PHE	50 ml	17-0147-01
	200 ml	17-0147-02
	1 l	17-0147-04
	5 l	17-0147-05

For local office contact information, visit
www.gelifesciences.com/contact

www.gelifesciences.com/protein-purification

GE Healthcare Bio-Sciences AB
Björkgatan 30
751 84 Uppsala
Sweden

imagination at work

GE Healthcare UK Limited
Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA, UK

GE Healthcare Europe, GmbH
Munzinger Strasse 5, D-79111 Freiburg, Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327, Piscataway, NJ 08855-1327, USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho, Shinjuku-ku, Tokyo 169-0073, Japan

18-1128-86 AC 03/2009