Tricorn MonoBeads

Mono Q 5/50 GL, Mono Q 4.6/100 PE, Mono Q 10/100 GL, Mono Q HR 16/10, Mono Q PC 1.6/5, Mono S 5/50 GL, Mono S 4.6/100 PE, Mono S 10/100 GL, Mono S HR 16/10, Mono S PC 1.6/5, Mono P 5/50 GL, and Mono P 5/200 GL

ION EXCHANGE CHROMATOGRAPHY

MonoBeads™ prepacked columns are designed for high performance, laboratory scale separations of proteins and other biomolecules according to charge or isoelectric point, using ion exchange chromatography or chromatofocusing. MonoBeads offer high resolution purification at high capacity with reproducible results, which makes MonoBeads prepacked columns highly suitable for use during the polishing step where remaining trace contaminants are removed.

- Monodisperse porous beads for high resolution purification and high capacity
- Highly suitable for the polishing step when high level purity is required
- Separation of proteins and other biomolecules according to charge or isoelectric point
- Excellent reproducibility and durability

Fig 1. Tricorn columns prepacked with MonoBeads for high resolution ion exchange and chromatofocusing.

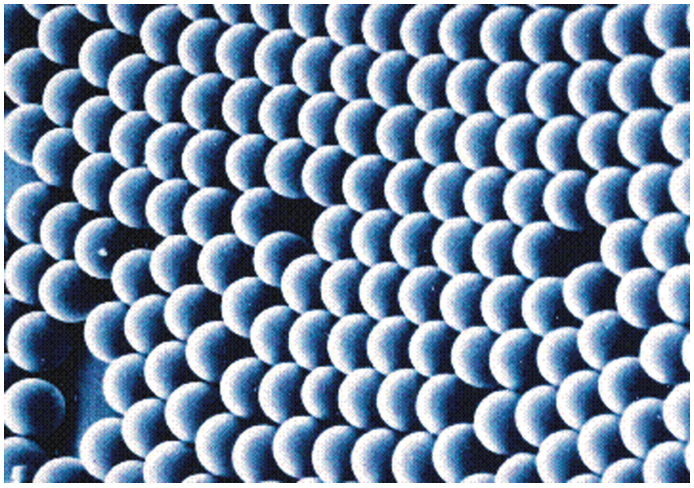


Fig 2. An electron micrograph of the beaded polystyrene/divinyl benzene MonoBeads shows distinct monodispersity. This allows highly efficient operation for Mono Q, Mono S, and Mono P at medium pressures.

Media characteristics

MonoBeads prepacked columns are available with three different media: Mono Q™, Mono S™ or Mono P™.

Table 1 summarizes the main characteristics of MonoBeads.

Monodisperse porous beads for high resolution and high capacity

MonoBeads is a beaded polystyrene/divinyl benzene medium (Fig 2). High efficiency is obtained from the use of small, spherical, monodispersed beads (10 µm), expertly packed in high performance columns. This high efficiency, coupled with the excellent selectivity of the Q, S, and P substituents, results in high resolution separation.

MonoBeads media are porous, with charged surfaces within the beads as well as on the outside. This gives MonoBeads a high binding capacity, particularly for Mono Q and Mono S, which can bind up to 50 mg globular protein/ml medium. Mono P columns can bind up to 10 mg globular protein/ml medium.

MonoBeads media are based on highly rigid beads, which means that they can be used at high flow rates, with retained function and stability.

Highly suitable for the polishing step

A protein purification procedure often consists of three distinct steps – capture, intermediate purification, and polishing. The capture step aims at concentrating and stabilizing the target protein while intermediate purification removes most of the bulk impurities. Polishing provides the final purity, eliminating trace contaminants and/or closely related substances.

MonoBeads offer high resolution, high capacity and high flow rates. This makes MonoBeads highly suitable for polishing when high level purity is essential, and an appropriate choice for intermediate purification.

Separation of proteins and biomolecules according to charge or isoelectric point

Ion exchange separations exploit the different charge characteristics of amino acids and other components on the surface of biomolecules. Separation is based on the reversible interaction between the charged biomolecule and oppositely charged molecules on the chromatographic medium. Mono Q and Mono S are strong anion and cation exchangers, respectively. Used in this context "strong" means that the ion exchange properties of the medium remain constant during chromatography within the recommended pH range of 2–12. This stability allows flexibility in choosing the best working pH for a specific sample. Elution is usually achieved by changing the buffer ionic strength (with a salt gradient), but can also be achieved by changing the net charge of the bound compounds (with a pH gradient).

In contrast, Mono P is a weak anion exchanger with a precisely balanced "mixture" of charged groups of different pK_a values that give the medium its buffering properties. Mono P is used in combination with special buffers, Polybuffer™ or Pharmalyte™, which form a pH gradient in the column. As the gradient develops, components elute, usually in the order of their isoelectric points. This separation method is known as chromatofocusing. Resolution is excellent and the peak widths can be as narrow as 0.02 pH units. Chromatofocusing thus combines the high resolution of analytical isoelectric focusing with the simplicity and convenience of preparative chromatography.

High chemical and pH stabilities

The chemistry of Mono Q, Mono S, and Mono P provides excellent performance benefits. MonoBeads are very pH-stable and can therefore be operated at optimal pH for separation and can be cleaned effectively. The media are stable for continuous use in the pH range 2–12, although pH values as high as 14 can be used during cleaning and sanitizing procedures. MonoBeads can be used with solutions of most buffers used in biochemical separations of biomolecules, and in water-alcohol (C1–C4) and acetonitrile-water solutions. The resistance of MonoBeads to organic solvents allows for complete cleaning and the use of conditions necessary for the solubilization of very hydrophobic samples. Aqueous solutions of guanidine hydrochloride, isopropanol, and similar compounds can be used as alternative cleaning solutions. Nonionic detergents, zwitterionic detergents, or detergents with the same charge as the ion exchange groups may be used. Oxidizing agents should be avoided. For more information see Table 1.

Column characteristics

MonoBeads are prepacked in high performance Tricorn™, HR, and Precision columns for reproducibility and durability. All parts of the columns are biocompatible.

Tricorn high performance columns

The design of Tricorn columns gives high performance without compromising user friendliness and reliability. The liquid is distributed evenly over the entire column cross-section to enable high resolution separations. The columns are simple to use, with Valco™ fittings for easy connection to ÄKTAdesign™ and other high performance LC systems, and can be run according to their specifications where the systems have the appropriate pressure capacity.

In Tricorn GL columns the tube is made of glass, which allows for visual inspection of the media bed. Tricorn GL columns are coated with a protective plastic film to protect the tube and for personal safety. A moveable adapter simplifies maintenance and a locking ring protects the media bed from compression by accidental turning of the adapter. Tricorn columns are robust and simple both to operate and maintain.

Mono Q and Mono S are also available prepacked in Tricorn PE columns, in which the tube and filter are made of PEEK (polyetheretherketone).

HR columns

HR 16/10 columns are high performance glass columns with larger bed volumes than Tricorn columns, which give them higher loading capacity. HR columns can be used with ÄKTAdesign systems and other high performance chromatography systems by using the connector that is supplied with the column.

Precision columns

PC 1.6/5 columns are designed for micropurification of subnanogram to microgram amounts of sample. These columns are recommended for use with the Ettan™ LC System, using the Precision Column Holder. For optimum results, it is important to check the compatibility of the column and the system specifications.

Excellent reproducibility and durability

Reproducible results are essential in all research. The long working life and high reproducibility (Fig 3) of MonoBeads prepacked columns, both run-to-run and column-to-column, are a result of optimized column design, the stable nature of MonoBeads, and well-proven, reliable column packing procedures. These are in addition to the controlled column manufacturing and media synthesis, and strict quality control testing of every production batch of MonoBeads and each prepacked column. Properly maintained MonoBeads prepacked columns can be used for up to several thousand runs.

Table 1. Characteristics of MonoBeads

Properties	**Mono Q**	**Mono S**	**Mono P**
Type of ion exchanger	Strong anion	Strong cation	Chromatofocusing (weak anion exchanger)
Matrix	Monodisperse porous polystyrene/divinyl benzene beads	Monodisperse porous polystyrene/divinyl benzene beads	Monodisperse porous polystyrene/divinyl benzene beads
Charged group	$-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-N^+(CH_3)_3$	$-O-CH_2-CHOH-CH_2-O-CH_2-CHOH-CH_2-SO_3^-$	Mixed quaternary and tertiary amines
Ionic capacity per ml packed medium	0.27–0.37 mmol Cl^-	0.12–0.15 mmol H^+	0.15–0.21 mmol Cl^-
Chemical stability	Daily use: All commonly used aqueous buffers, pH 2–12 Urea, up to 8 M; acetonitrile, up to 20% in aqueous buffers; nonionic detergents; cationic detergents (Mono Q); anionic detergents (Mono S) Cleaning: Acetonitrile, up to 20%; sodium hydroxide, up to 2 M; ethanol, up to 100%; methanol, up to 100%; acetic acid, up to 75%; isopropanol, up to 100%; hydrochloric acid up to 1M, guanidine hydrochloride, up to 6 M; Avoid: Oxidizing agents; anionic detergents (Mono Q); cationic detergents (Mono S)		
pH stability range*			
long term	2–12	2–12	2–12
short term	2–14	2–14	2–14
Temperature range			
Working	4 to 40 °C	4 to 40 °C	4 to 40 °C
Storage	4 to 30 °C	4 to 30 °C	4 to 30 °C
Bead size	10 µm	10 µm	10 µm

* pH stability: Long term refers to the pH range over which the ion exchange groups remain charged and maintain consistently high capacity. Short term refers to the pH interval for regeneration and cleaning procedures.

Column: Mono S 5/50 GL (Tricorn)
Sample: Ribonuclease A (1.5 mg/ml), Cytochrome C (0.4 mg/ml)
Lysozyme (0.4 mg/ml)
Sample load: 200 ml
Flow rate: 1.0 ml/min
Buffer A: 20 mM sodium phosphate pH 6.8
Buffer B: 20 mM sodium phosphate pH 6.8 + 0.4 NaCl
Gradient: 0-100% B, 20 CV
System: ÄKTAFPLC™
Detection: A_{280nm}

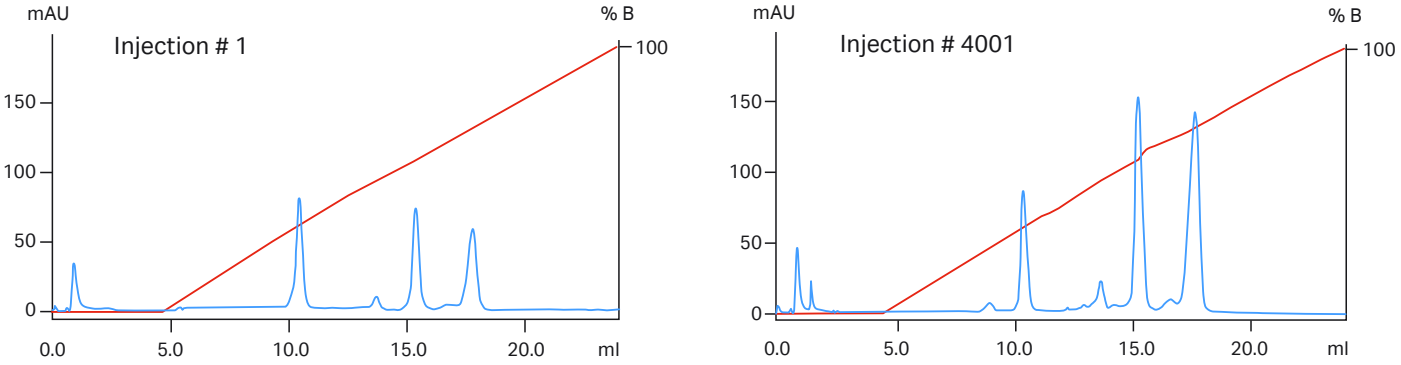


Fig 3. Long term reproducibility test on Mono S 5/50 GL. The chromatograms show the 1st and 4001st separations from a series of runs with a test sample on the same column. After 4000 runs the chromatographic performance is retained.

Table 2. Characteristics of MonoBeads prepacked columns

	Tricorn columns					HR columns	Precision columns
	Mono Q 5/50 GL Mono S 5/50 GL	Mono Q 10/100 GL Mono S 10/100 GL	Mono Q 4.6/100 PE Mono S 4.6/100 PE	Mono P 5/50 GL	Mono P 5/200 GL	Mono Q HR 16/10 Mono S HR 16/10	Mono Q PC 1.6/5 Mono S PC 1.6/5
Bed dimensions (i.d. × bed height)	5 × 50 mm	10 × 100 mm	4.6 × 100 mm	5 × 50 mm	5 × 200 mm	16 × 100 mm	1.6 × 50 mm
Bed volume	1 ml	8 ml	1.7 ml	1 ml	4 ml	20 ml	0.1 ml
Max loading capacity[1]	50 mg	400 mg	85 mg	10 mg	40 mg	1000 mg	5 mg
Tube material[2]	Glass	Glass	PEEK	Glass	Glass	Glass	Glass
Filter material[2]	PP	PP	PEEK	PP	PP	PP	Titanium
Flow rate recommended (maximum)	0.5–3.0 ml/min (3.0 ml/min)	2.0–6.0 ml/min (10.0 ml/min)	0.5–3.0 ml/min (3.0 ml/min)	0.5–1.5 ml/min (3.0 ml/min)	0.5–1.5 ml/min (2.0 ml/min)	– (10 ml/min)	0.01–0.4 ml/min (0.4 ml/min)
Maximum pressure over column	40 bar (580 psi, 4 MPa)	40 bar (580 psi, 4 MPa)	40 bar (580 psi, 4 MPa)	40 bar (580 psi, 4 MPa)	40 bar (580 psi, 4 MPa)	30 bar (435 psi, 3 MPa)	50 bar (725 psi, 5 MPa)

[1] Will vary depending on sample and loading conditions
[2] PEEK = polyetheretherketone; PP = polypropylene

Operation

Versatile purification possibilities

Mono Q, Mono S, and Mono P can be run under different combinations of pH, ionic strength, and gradient shape. This means that the purification protocol can be set up to exploit the charge characteristics of amino acids and other components on the surface of the biomolecules. For example, a separation optimized on MonoBeads can resolve amino acids, charged saccharides, and nucleotides that differ by as little as one net charge.

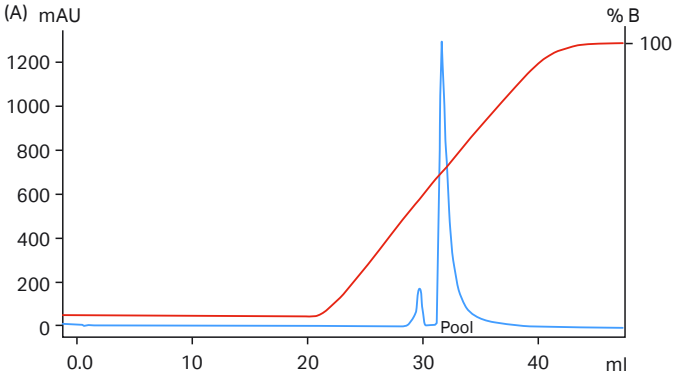
Applications

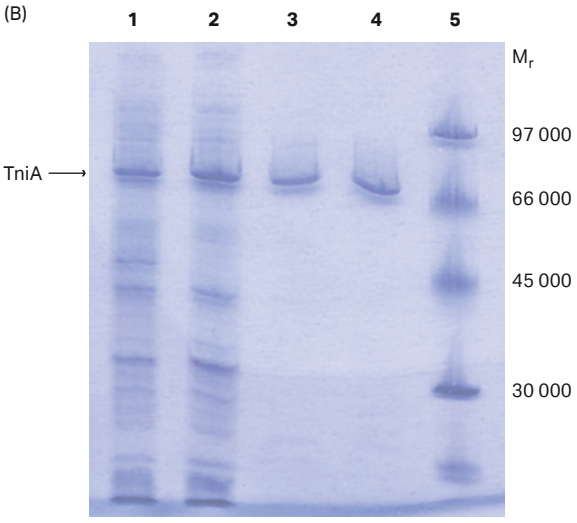
The high resolution, high capacity, and high flow rates, as well as the versatile purification possibilities and excellent reproducibility offered by MonoBeads make the columns very useful in many different kinds of applications. Thousands of published references describe the successful use of Mono Q, Mono S, and Mono P columns. The example shown below illustrates one application area.

Purification of recombinant transposase TniA

Transposase enzymes are impressive by their ability to perform many actions: they recognize and bind the ends of a transposon (a gene element), cleave it off, and then incorporate it into the target DNA. To prepare transposase TniA for characterization, a three-step purification was developed. Initial purification was performed on SOURCE™ 15Q 4.6/100 PE and HiTrap™ Heparin HP. The final polishing purification step was performed on Mono S 5/50 GL to obtain high purity (Fig 4).

Column:	Mono S 5/50 GL (Tricorn)
Sample:	Recombinant transposase TniA partially purified on SOURCE 15Q 4.6/100 and HiTrap Heparin HP, 5 ml
Sample load:	14.5 ml
Start buffer (A):	20 mM MES, 1 mM EDTA, 2 mM $MgCl_2$, 1 mM DTT at pH 6.5
Elution buffer (B):	A + 1M NaCl
Flow rate:	1 ml/min
Gradient:	0–100% elution buffer in 20 CV
System:	ÄKTAexplorer™ 100
Detection:	$A_{280\ nm}$





Lane 1: Sample, clarified extract diluted 5-fold
Lane 2: Pooled from SOURCE 15Q 4.6/100 PE
Lane 3: Pooled from HiTrap Heparin
Lane 4: Pooled from Mono S 5/50 GL
Lane 5: LMW-SDS Marker Kit

Fig 4. (A) Purification of recombinant transposase TniA on Mono S 5/50 GL (Tricorn). The chromatogram shows two highly resolved peaks with TniA in the larger peak. (B) Analysis of the three purification step using SDS-PAGE PhastGel™ Homogenous — 12.5, PhastSystem™, Coomassie™ stained.

Ordering information

Product	Quantity	Code number
Mono Q 5/50 GL	1	17-5166-01
Mono Q 10/100 GL	1	17-5167-01
Mono Q 4.6/100 PE	1	17-5179-01
Mono Q HR 16/10	1	17-0506-01
Mono Q PC 1.6/5*	1	17-0671-01
Mono S 5/50 GL	1	17-5168-01
Mono S 10/100 GL	1	17-5169-01
Mono S 4.6/100 PE	1	17-5180-01
Mono S HR 16/10	1	17-0507-01
Mono S PC1.6/5*	1	17-0672-01
Mono P 5/50 GL	1	17-5170-01
Mono P 5/200 GL	1	17-5171-01
Mono Q HR 16/10	1	17-0506-01
Mono S HR 16/10	1	17-0507-01

* PC columns require the use of Precision Column Holder when used with standard chromatography systems

Related products

Product	Quantity	Code number
Mini Q™ 4.6/50 PE	1	17-5177-01
Mini S™ 4.6/50 PE	1	17-5178-01
SOURCE 15Q 4.6/100 PE	1	17-5181-01
SOURCE 15S 4.6/100 PE	1	17-5182-01
Pharmalyte, broad range pH 8–10.5	25 ml	17-0455-01
Polybuffer 74	250 ml	17-0713-01
Polybuffer 96	250 ml	17-0714-01
Precision Column Holder	1	17-1455-01

Related product information

Ion Exchange Chromatography & Chromatofocusing: Principles and Methods	11-0004-21
Protein Purification, Handbook	18-1132-29
Prepacked chromatography columns for ÄKTAdesign and ETTAN LC Systems, Selection guide	28-9317-78
Ion exchange columns and media, Selection guide and product profile	18-1127-31

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