instructions

Arginine Sepharose 4B

Arginine Sepharose™ 4B is L-arginine immobilized to Sepharose 4B by an epoxy coupling method via a long hydrophilic spacer and stable ether and alkylamine bonds. L-arginine is coupled via its α-amino group, leaving the guanidino and α-carboxyl groups free to interact with sample substances during chromatography.

Arginine Sepharose 4B is designed for purification of molecules with bio-specific or charge dependent affinity for L-arginine.

Both electrostatic and stereospecific effects may contribute to the separation depending upon the application.

71-7101-00 Edition AC

Amersham Biosciences

Arginine Sepharose 4B has been used to isolate or remove a number or different serine proteases, *e.g.* prekallikrein, clostripain, plasminogen activator, prothrombin and maturation promoting factor, from a wide range of starting materials.

Table 1. Medium characteristics.

Ligand density:	14–20 µmol arginine/ml drained medium
Binding capacity:	No data available
Bead structure:	4% agarose
Mean particle size:	90 µm
Bead size range:	45–165 µm
Max linear flow rate*:	75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH stability**	
Long term:	2–13
Short term:	2–13
Chemical stability:	Stable to all commonly used aqueous buffers
Physical stability:	Negligible volume variation due to changes in pH or ionic strength.

* Linear flow rate = $\frac{\text{volumetric flow rate (cm}^3\text{/h)}}{\text{Column cross-sectional area (cm}^2\text{)}}$

** The ranges given are estimates based on our knowledge and experience. Please note the following:
pH stability, long term refers to the pH interval where the medium is stable over a long period of time without adverse effects on its subsequent chromatographic performance.
pH stability, short term refers to the pH interval for regeneration.

Preparation of the medium

Arginine Sepharose 4B is supplied pre-swollen in 20% ethanol. Prepare a slurry by decanting the ethanol solution and replacing it with binding buffer in a ratio of 75% settled medium to 25% buffer before packing. The binding buffer should not contain agents which significantly increase the viscosity. The column may be equilibrated with viscous buffers at reduced flow rates after packing is completed.

Packing Sepharose 4B

1. Equilibrate all material to the temperature at which the chromatography will be performed.
2. De-gas the medium slurry.
3. Eliminate air from the column dead spaces by flushing the end pieces with buffer. Make sure no air has been trapped under the column net. Close the column outlet with a few centimeters of buffer remaining in the column.
4. Pour the slurry into the column in one continuous motion. Pouring the slurry down a glass rod held against the wall of the column will minimize the introduction of air bubbles.
5. Immediately fill the remainder of the column with buffer, mount the column top piece onto the column and connect the column to a pump.
6. Open the bottom outlet of the column and set the pump to flow at the desired flow rate. This should be at least 133% of the flow rate to be

used during subsequent chromatographic procedures. However, the maximum flow rate, see table 1, is typically employed during packing.

Note: Do not exceed 75% of the packing flow rate in subsequent chromatographic procedures.

7. Maintain the packing flow rate for 3 bed volumes after a constant bed height is reached.

Using an adaptor

Adaptors should be fitted as follow:

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully add more buffer into the column to form an upward meniscus.
2. Insert the adaptor at an angle into the column, ensuring that no air is trapped under the net.
3. Make all tubing connections at this stage. There must be a bubble-free liquid connection between the column and the pump.
4. Slide the plunger slowly down the column so that the air above the net and in the capillary tubings is displaced by eluent. Valves on the inlet side of the column should be turned in all directions during this procedure to ensure that air is removed.
5. Lock the adaptor in position on the medium surface, open the column outlet and start the eluent flow. Pass eluent through the column at the packing flow rate until the medium bed is stable. Re–positioning the adaptor on the medium surface as necessary.

The column is now equilibrated and ready for use.

Binding

Different substances differ in their affinity for Arginine Sepharose 4B. The available capacity will depend upon parameters such as sample concentration, flow rate, pH, buffer composition and temperature. To obtain an optimal purification with respect to capacity, the capacity must first be determined over a range of different pH and flow rates.

1. Sample pH should be the same as that of the binding buffer. Filter the sample through a 0.22 µm or 0.45 µm filter to prolong the working life of the medium.
2. After the sample has been loaded, wash the medium with binding buffer until the baseline is stable.

Elution

Arginine Sepharose 4B is a group specific adsorbent with affinity for a variety of biomolecules. Some proteins interact biospecifically due to its structural similarity with the ligand while others, bind in a less specific manner by electrostatic interactions.

- Specifically bound biomolecules may be eluted by competitive elution. Use of a competing agent for either the ligand or the target molecule, *e.g.* arginine in the buffer will elute specifically bound substances.
- Less specifically bound biomolecules can be eluted with increased ionic strength. Elution is normally complete at salt concentrations of 2 M

or less of NaCl. Either step or continuous gradients may be used.

- Reduction of the polarity of the elution buffer by addition of dioxane (up to 10%) or ethylene glycol (up to 50%) may be used for elution of bound substances.
- An alternative for elution is to use urea or guanidine hydrochloride which act as deforming agents and elute bound substances.

Regeneration

Depending of the nature of the sample, Arginine Sepharose 4B may be regenerated for re-use by washing the medium with 2–3 bed volumes of alternating high pH (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and low pH (0.1 M sodium acetate, 0.5 M NaCl, pH 4.5) buffers. This cycle should be repeated 3 times followed by re-equilibration with at least 5 bed volumes of binding buffer.

Strongly adsorbed proteins can be removed by including 8 M urea or 6 M guanidine hydrochloride in the regeneration buffer.

Strongly adsorbed proteins may also be removed by washing with 2–3 bed volumes 0.5 M NaOH immediately followed by re-equilibration with at least 5 bed volumes of binding buffer.

An alternative method for regeneration of the medium is to wash the medium with a detergent

solution, *e.g.* 0.1% Triton™ X-100 at 37 °C for one minute. Re-equilibrate immediately with at least 5 bed volumes of binding buffer. If necessary, 8 M urea or 6 M guandine hydrochloride may be added to the detergent solution.

Storage

For longer periods of storage, keep Arginine Sepharose at 4–8 °C in a suitable bacteriostat, *e.g.* 20% ethanol. The medium must not be frozen.

Further information

Check www.chromatography.amershambiosciences.com for more information. Useful information is also available in the Affinity Chromatography Handbook, see ordering information.

Ordering Information

Product	Pack size	Code No.
Arginine Sepharose 4B	25 ml	17-0524-01
Literature		
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

Trademarks

Sepharose and Drop Design are trademarks of Amersham Biosciences Limited. Amersham and Amersham Biosciences are trademarks of Amersham plc. Triton is a trademark of Union Carbide Chemicals and Plastics Co.

All goods and services are sold subject to the terms and conditions of sale of the company within the Amersham Biosciences group which supplies them. A copy of these terms and conditions is available on request.

Amersham Biosciences AB
SE-751 84 Uppsala Sweden

Amersham Biosciences UK Limited
Amersham Place, Little Chalfont
Buckinghamshire, England HP7 9NA

Amersham Biosciences Corporation
800 Centennial Ave
Piscataway, NJ 08855 USA

Amersham Biosciences Europe GmbH
Postfach 5480
D-79021 Freiburg Germany

Amersham Biosciences K.K.
Sanken Building, 3-25-1
Shinjuku-ku, Tokyo 169-0073 Japan

Produced by Wikströms, Sweden 1021352, 10.2002
Printed matter. Licence 341 051

Amersham Biosciences