instructions

5’ AMP Sepharose 4B

5' AMP Sepharose™ 4B interacts strongly with NAD^+-dependent dehydrogenases and ATP-dependent enzymes. Selective elution with gradients of NAD^+ or $NADP^+$ has allowed the resolution of complex mixtures of dehydrogenase isoenzymes using 5’ AMP Sepharose 4B. 5’ AMP Sepharose 4B is synthesized in several steps. Diaminohexane is linked to AMP via the N6 of the purine ring. The derivatised AMP is then immobilized to Sepharose 4B via the aminohexane spacer.

71-7091-00 Edition AC

Amersham Biosciences

Table 1. Medium characteristics.

Ligand density:	approx. 2 µmole 5' AMP/ml drained medium
Available capacity*:	approx. 10 mg lactate dehydrogenase/ml drained medium
Bead structure:	4% agarose
Bead size range:	45–165 µm
Mean bead size:	90 µm
Max linear flow rate**:	75 cm/h at 25 °C, HR 16/10 column, 5 cm bed height
pH stability***	
Long term:	4–10
Short term:	4–10
Chemical stability:	Stable to all commonly used aqueous buffers and additives like detergents. Avoid high concentrations of EDTA, urea, guanidine-HCl, chaotropic salts and strong oxidizing agents
Physical stability:	Negligible volume variation due to changes in pH or ionic strength

* The capacity data were determined in 0.1 M phosphate buffer, pH 7.0

** 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 and cleaning.

Preparing the medium

5' AMP Sepharose 4B is supplied freeze-dried in the presence of additives. These additives must be washed away at neutral pH.

Weigh out the required amount of freeze dried powder (1 g freeze-dried powder gives 3.5–5 ml final medium volume) and suspend it in distilled water. The medium swells immediately and should now be washed for 15 minutes with distilled water on a sintered glass filter. Use approximately 200 ml distilled water per gram freeze-dried powder, added in several aliquots.

Prepare a slurry with binding buffer, in a ratio of 75% settled medium to 25% buffer. 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 run 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: If you have packed at the maximum linear flow rate, do not exceed 75% of this 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 follows:

1. After the medium has been packed as described above, close the column outlet and remove the top piece from the column. Carefully fill the rest of the column with buffer to form an upward meniscus at the top.
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 bed is stable. Re-position the adaptor on the medium surface as necessary.

The column is now packed and equilibrated and ready for use.

Binding

Proteins which bind to 5' AMP Sepharose 4B do so around physiological pH. To avoid non-specific interactions with the medium, we recommend using a buffer with an ionic strength of at least 0.15 M.
A recommended binding buffer is 10 mM phosphate buffer, pH 7.3, containing 0.15 M NaCl.

If the protein of interest binds to the medium by ionic forces, a binding buffer of lower ionic strength may be necessary.
After sample has been loaded, wash the medium with binding buffer until the base line is stable.

Elution

5' AMP Sepharose 4B is a group specific adsorbent with affinity for a variety of biomolecules. Some proteins interact biospecifically due to their structural similarity with the ligand while others bind in a less specific manner by electrostatic and/or hydrophobic interactions.

- Specifically bound biomolecules may be eluted by competitive elution with low concentrations of NAD^+ or $NADP^+$ in the buffer. Specifically bound biomolecules normally elute in the range 1–20 mM. Either step or continuous gradients may be used.
- Less specifically bound biomolecules can be eluted with increased ionic strength. Elution is normally complete at salt concentrations of 1 M or less of NaCl. Either step or continuous gradients may be used. Elution can also be achieved by a change in pH.

Regeneration

Depending of the nature of the sample, 5' AMP 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 3–5 bed volumes of binding buffer.

If detergent or denaturing agents (*e.g.* 6 M urea) have been used during chromatography, these can also be used in the washing buffer.

Cleaning

In some applications, substances like denaturated proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the column 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.

Storage

Freeze-dried 5' AMP Sepharose 4B should be kept dry and stored below 8 °C.

Swollen medium should be stored in neutral pH at 4–8 °C in presence of a bacteriostat, *e.g.* 20% ethanol. Exposure to solutions with pH greater than 10 may cause loss of phosphate groups. The swollen medium must not be frozen.

Ordering information

Product	Pack size	Code No.
5’ AMP Sepharose 4B	5 g	17-0620-01
Literature		
Affinity Chromatography Handbook, Principles and Methods	1	18-1022-29

Further information

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

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 1021120, 09.2002
Printed matter. Licence 341 051

Amersham Biosciences