cytiva

# 2′5′ ADP **Sepharose** 4B

## Affinity chromatography

## Instructions for Use

2′5′ ADP Sepharose™ 4B interacts strongly with $NADP^+$-dependent dehydrogenases. Selective elution with gradients of $NAD^+$ or $NADP^+$ has allowed the resolution of complex mixtures of dehydrogenase isoenzymes using 2′5′ ADP Sepharose 4B.

2′5′ ADP Sepharose 4B is synthesized in several steps. Diaminohexane is linked to 2′5′ ADP via the N6 of the purine ring. The derivatized ADP is then immobilized to Sepharose 4B via the aminohexane spacer.

# Table of contents



**Important**

Read these instructions carefully before using the product.

**Safety**

For use and handling of the product in a safe way, refer to the safety Data Sheet.

# 1 Media characteristics

**Table 1.** Medium characteristics.

| | |
|---|---|
| **Matrix** | 4% agarose |
| **Ligand density** | approx. 2 µmol 2'5' ADP/ml drained medium |
| **Average particle size** | 90 µm |
| **Available binding capacity**[1] | approx. 0.4 mg glucose-6-phosphate dehydrogenase/ml drained medium. |
| **Maximum flow velocity**[2] | 75 cm/h at 25ºC |
| **pH stability**[3]<br>- Working range<br>- Cleaning-in-place | <br>4 to 10<br>4 to 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. |

[1] The binding capacity was determined in 0.1 M Tris-HCl, 5 mM EDTA, 1 mM 2-mercaptoethanol, pH 7.6.

[2] $H_2O$ in a 1.6 × 10 cm (i.d. × H) column, bed height 5 cm.

[3] Working range: pH interval where the medium can be operated without significant change in function.

Cleaning-in-place: pH stability where the medium can be subjected to cleaning-in-place without significant change in function.

# 2 Preparing the medium

2′5′ ADP 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 to 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.

# 3 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 velocity, see Table 1, is typically employed during packing.

   ***Note:*** *If you have packed at the maximum flow velocity, 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.

# 4 Using an adapter

Adapters 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 adapter 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 adapter 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 is ready for use.

# 5 Elution

2′5′ ADP 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 to 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 or by temperature gradients.

# 6 Regeneration

Depending of the nature of the sample, 2'5' ADP Sepharose 4B may be regenerated for re-use by washing the medium with alternating 2 to 3 bed volumes of high pH (0.1 M Tris-HCl, 0.5 M NaCl, pH 8.5) and 2 to 3 bed volumes of 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 to 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.

# 7 Cleaning

In some applications, substances like denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by washing the column with a detergent solution at 37°C for one minute.

Re-equilibrate immediately with at least 5 bed volumes of binding buffer.

# 8 Storage

Freeze-dried 2′5′ ADP Sepharose 4B should be kept dry and stored below 8°C. Swollen medium should be stored in neutral pH at 2°C to 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.

# 9 Further information

Check cytiva.com/protein-purification for more information. Useful information is also available in the Affinity Chromatography Handbook, see ordering information.

# 10 Ordering information

| Product | Pack size | Code No. |
|---|---|---|
| 2′5′ADP Sepharose 4B | 5 g | 17-0700-01 |

| Related literature | Pack size | Code No. |
|---|---|---|
| Affinity Chromatography Handbook, Principles and Methods | 1 | 18-1022-29 |

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