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Dextrin **Sepharose** High Performance

Affinity resin

Instructions for Use

Dextrin Sepharose™ High Performance is a chromatography medium for purifying proteins tagged with maltose binding protein (MBP). Thanks to the high specificity of the binding, very high purity is achieved in just one step.

Recombinant proteins are engineered with MBP-tags to facilitate detection, isolation and purification procedures. In addition, the MBP-tag is often chosen due to its ability to increase the expression level and solubility of the fusion protein. Purification of MBP-tagged protein is done under physiological conditions, which together with mild elution by maltose, preserves the activity of the target protein.

Dextrin Sepharose High Performance is available in 25 and 100 ml lab packs and prepacked in 1 and 5-ml MBPTrap™ HP columns.

Safety

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

Contents

1 Description

This robust, high-resolution chromatography medium is based on the 34 µm Sepharose High Performance matrix. Due to the small size of the beads, the MBP-tagged protein is eluted in a narrow peak, minimizing the need for further concentration steps.

Purification is performed under physiological conditions and mild elution using maltose preserves the activity of the target protein. These mild conditions may even allow purification of intact protein complexes.

Dextrin Sepharose High Performance tolerates all commonly used aqueous buffers and is easily regenerated using 0.5 M sodium hydroxide.

Table 1 summarizes the characteristics of the Dextrin Sepharose High Performance medium.

Table 1. Dextrin Sepharose High Performance medium characteristics.

Matrix	Rigid, highly cross-linked 6% agarose
Average particle size	34 µm
Ligand	Dextrin
Dynamic binding capacity[1]	Approx. 7 mg MBP2*-paramyosin ΔSal/ml medium (M_r ~70 000, multimer in solution) Approx. 16 mg MBP2*-ß galactosidase/ml medium (M_r ~158 000, multimer in solution)
Max. linear flow rate[2]	300 cm/h
Recommended flow rate[2]	≤ 150 cm/h
Maximum back pressure[2]	0.3 MPa, 3 bar
Chemical stability[3]	Stable in all commonly used aqueous buffers, 0.5 M NaOH (regeneration and cleaning)
pH stability	Working range: > 7 Short-term: 2–13
Storage	4 to 8°C in 20% ethanol

[1] Dynamic binding capacity (DBC) is defined as mg protein applied per ml medium at the point where the concentration of protein in the column effluent reaches a value of 10% of the concentration in the sample. Binding capacity is protein dependent.

[2] H_2O at room temperature.

[3] The presence of reducing agents, e.g. 5 mM DTT, may decrease yield. Higher ionic strength does not decrease affinity since MBP binds to dextrin primarily by hydrogen binding. Agents that interfere with hydrogen binding, such as urea and guanidine hydrochloride, are not recommended. The presence of 10% glycerol may decrease the yield and 0.1% SDS completely eliminates the binding.

2 General considerations

Recombinant proteins are engineered with MBP-tags to facilitate detection, isolation and purification procedures. In addition, the MBP-tag is often chosen due to its ability to increase the expression level and solubility of the fusion protein.

Purification of MBP-tagged protein is done under physiological conditions, which together with mild elution by maltose, preserves the activity of the target protein. These mild conditions may even allow purification of intact protein complexes.

Regeneration is fast and easy to perform using 0.5 M NaOH, which is also used for cleaning the column.

As an alternative, 0.1% SDS can also be used for regeneration. SDS completely eliminates the binding of MBP to dextrin.

3 Column packing

Dextrin High Performance is supplied preswollen in 20% ethanol.

Prepare a slurry by decanting the 20% ethanol solution and replacing it with distilled water in a ratio of 75% settled medium to 25% distilled water. Water is used as packing solution.

Table 2. Recommended lab-scale columns for Dextrin Sepharose High Performance

Empty Column[1]	Packing flow rate[2] (ml/min)		Recommended flow rate[2] for chromatography
	First step	Second step	(ml/min)
Tricorn™ 5/20	0.5	1	0.5
Tricorn 5/50	0.5	1	0.5
Tricorn 10/20	2	4	2
Tricorn 10/50	2	4	2
Tricorn 10/100	2	4	2
XK 16/20	5	10	5
XK 26/20	13	27	13

[1] For inner diameter and maximum bed volumes and bed heights, see Section 9.

[2] The recommended flow rates equals a linear flow rate of approximately 150 cm/h.

Packing protocol

1. Assemble the column (and packing reservoir if necessary).
2. Remove air from the end-piece and adapter by flushing with water.
   Make sure no air has been trapped under the column bed support. Close the column outlet leaving the bed support covered with water.
3. Resuspend the medium and pour the slurry into the column in a single continuous motion. Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles.
4. If using a packing reservoir, immediately fill the remainder of the column and reservoir with water. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.
5. Open the bottom outlet of the column and set the pump to run at the desired flow rate, see Table 2 or below. It is recommended to pack Sepharose High Performance chromatography media in XK or Tricorn columns in a two-step procedure. Do not exceed 1.0 bar (0.1 MPa) in the first step and 3.5 bar (0.35 MPa) in the second step.
   - If the packing equipment does not include a pressure gauge, use a first step packing flow rate of 5 ml/min (XK 16/20 column) or 2 ml/min (Tricorn 10/100 column), and a second step packing flow rate of 9 ml/min (XK 16/20 column) or 3.6 ml/min (Tricorn 10/100 column). See Table 2 for packing flow rates for other columns.
   - If the recommended pressure or flow rate cannot be obtained, use the maximum flow rate your pump can deliver. This should also give a well packed bed.

   Note: *For subsequent chromatography procedures, do not exceed 75% of the packing flow rate. See Table 2 for flow rates for chromatography.*

6 Maintain packing flow rate for at least 3 bed volumes after a constant bed height is reached. Mark the bed height on the column.

7 Stop the pump and close the column outlet.

8 If using a packing reservoir, disconnect the reservoir and fit the adapter to the column.

9 With the adapter inlet disconnected, push the adapter down into the column until it reaches the mark. Allow the packing solution to flush the adapter inlet. Lock the adapter in position.

10 Connect the column to a pump or a chromatography system and start equilibration. Re-adjust the adapter if necessary.

4 Operation

Buffer preparation

Use high purity water and chemicals for buffer preparation. Filter buffers through a 0.22 µm or a 0.45 µm filter before use.

Recommended buffers

Binding buffer	20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, pH 7.4 Optional: 1 mM DTT
Elution buffer	10 mM maltose in binding buffer
Regeneration buffer	0.5 M NaOH or 0.1% SDS

Sample preparation

Adjust the sample to the composition of the binding buffer. For example, dilute the sample with binding buffer or buffer exchange using HiTrap™ Desalting, HiPrep™ 26/10 Desalting or Desalting PD-10 column, see Table 3.

To avoid clogging the column when loading large sample volumes, filter the sample through a 0.45 µm filter or centrifuge it immediately before application.

Purification

Recommended linear flow rate is 150 cm/h.

1. Remove the stoppers and connect the column to the system. Avoid introducing air into the column.
2. If the column has been stored in 20% ethanol, wash out the ethanol with at least 5 column volumes (CV) of distilled water or binding buffer at a linear flow rate of 50-100 cm/h.
3. Equilibrate the column with at least 5 CV of binding buffer.
4. Apply the pretreated sample. A lower flow rate can be used during sample application to optimize performance.
5. Wash with 5 to 10 CV of binding buffer or until no material appears in the effluent.
6. Elute with 5 CV of elution buffer. The eluted fractions can be buffer exchanged using a prepacked desalting column, see Table 3.

Regeneration and cleaning

Recommended linear flow rate is 75-150 cm/h.

1. Regenerate the column with 3 CV distilled water followed by 3 CV 0.5 M NaOH and 3 CV distilled water.
2. Re-equilibrate the column with 5 CV of binding buffer before starting the next purification.

Note: *An alternative to the above regeneration is to replace 0.5 M NaOH with 0.1% SDS. Do not regenerate with 0.1% SDS in a cold-room since the SDS may precipitate.*

Table 3. Prepacked columns for desalting and buffer exchange.

Column	Code No.	Loading volume	Elution volume	Comments	Application
HiPrep 26/10 Desalting	17-5087-01	2.5 to 15 ml	7.5 to 20 ml	Prepacked with Sephadex™ G-25 Fine. Requires a laboratory pump or a chromatography system to run.	For desalting and buffer exchange of protein extracts (M_r > 5000).
HiTrap Desalting	17-1408-01	0.25 to 1.5 ml	1.0 to 2.0 ml	Prepacked with Sephadex G-25 Superfine. Requires a syringe or pump to run.	
PD-10 Desalting	17-0851-01	1.0 to 2.5 ml [1] 1.75 to 2.5 ml [2]	3.5 ml [1] up to 2.5 ml [2]	Prepacked with Sephadex G-25 Medium. Runs by gravity flow or centrifugation	For desalting, buffer exchange, and cleanup of proteins and other large biomolecules (M_r > 5000).
PD MiniTrap™ G-25	28-9180-07	0.1 to 0.5 ml [1] 0.2 to 0.5 ml [2]	1.0 ml [1] up to 0.5 ml [2]		
PD MidiTrap™ G-25	28-9180-08	0.5 to 1.0 ml [1] 0.75 to 1.0 ml [2]	1.5 ml [1] up to 1.0 ml [2]		

[1] Volumes with gravity elution
[2] Volumes with centrifugation

5 Scale up

Scale-up is typically performed by keeping bed height and linear flow rate (cm/h) constant while increasing bed diameter and volumetric flow rate (ml/min).

6 Storage

Store Dextrin Sepharose High Performance in 20% ethanol at 4 to 8°C. After storage, equilibrate with binding buffer before use.

7 Troubleshooting

Fault	Possible cause/*corrective action*
Increased back pressure	High viscosity of solutions. • *Use lower flow rates* Insufficient cell disruption. • *Increase the efficiency of the mechanical cell disruption, e.g. increase sonication time. (Keep the sample on ice during sonication to avoid frothing and overheating as this may denature the target protein. Over-sonication can also lead to co-purification of host proteins with the target protein).* • *Increase dilution of the cell paste before mechanical lysis, or dilute after lysis to reduce viscosity.* • *If the lysate is very viscous due to a high concentration of host nucleic acid, continue sonication until the viscosity is reduced, and/or add additional DNAse. Alternatively, draw the lysate through a syringe needle several times.* • *If the purification has been performed at 4°C, try repeating it at room temperature if possible (sample viscosity is reduced at room temperature).* • *Decrease flow rate during sample loading.*
Increased back pressure	Freezing/thawing of the unclarified lysate has increased precipitation and aggregation. • *Sonicate the thawed lysate.*
Column has clogged	Top filter is clogged. • *Change top filter.* Cell debris in the sample may clog the column. • *Clean the column according to the section under Operation.* • *Centrifuge and/or filter the sample through a 0.22 µm or a 0.45 µm filter.*

Fault	Possible cause/*corrective action*
No or weak binding to the column	Protein found in the flow-through. • *Buffer/sample composition is not optimal; check the pH and composition of the sample and binding buffer. pH should in general be above pH 7.* Factors in the crude extract interfere with binding. • *Include glucose in the growth medium to suppress amylase expression.* MBP-tag is not present. • *Use protease-deficient* E. coli *expression strains. Add protease inhibitors during cell lysis.* MBP-tag is not accessible. • *Fuse the MBP-tag with the other protein terminus. Use another linker.* Protein has precipitated in the column due to high protein concentration. • *Clean the column according to instructions under Operation. In the following run decrease the amount of sample, or decrease protein concentration by eluting with a linear gradient instead of step-wise elution. Try detergents or change the NaCl concentration.*
Contaminating proteins	Contaminants are short forms of the tagged protein. • *Use protease deficient* E. coli *expression strains. Add protease inhibitors after cell lysis. Fuse the MBP-tag with the other protein terminus. Check for the presence of internal translation initiation starts (for C-terminal MBP-tag) or premature termination sites (for N-terminal MBP-tag). Use EDTA in the sample and buffers.* Contaminants are covalently linked to the recombinant protein via disulfide bonds. • *Add reducing agents to all buffers for cell lysis and purification. Note that the yield may decrease.* Contaminants are non-covalently linked to the recombinant protein. • *Increase ionic strength in all buffers for cell lysis and purification (up to 1 M NaCl) or add mild detergents (0.1% Triton™ X-100, 0.1% Tween™, 0.1% CHAPS). Be careful since the binding of MBP to dextrin may be affected by the addition of non-ionic detergents.*

Fault	Possible cause/*corrective action*
Unwanted air bubble formation	Unclarified lysates may increase air bubble formation during purification. • *Attaching a flow restrictor in the chromatography system can prevent this. If a flow restrictor is attached, it is important to change the pressure limit to adjust for the extra pressure from the flow restrictor. Do not exceed the pressure limit for the column on the ÄKTA™ system.* Air bubbles may form due to decreased air solubility when columns stored at 4 to 8°C are used immediately at room temperature. • *Let the columns adapt to room temperature for some minutes before using them.*

8 Further information

If you have further questions about Dextrin Sepharose High Performance, please visit:

- *cytiva.com/protein-purification*
- Cytiva technical support portal *cytiva.com/purification_techsupport*

or contact your local Cytiva representative.

For further information on expression, detection and/or assays for MBP-tagged proteins, see New England Biolabs.

9 Ordering information

Product	Pack size	Code No.
Dextrin Sepharose High Performance	25 ml	28-9355-97
Dextrin Sepharose High Performance	100 ml	28-9355-98

Related products	Pack size	Code No.
MBPTrap HP	5 × 1 ml	28-9187-78
MBPTrap HP	1 × 5 ml	28-9187-79
MBPTrap HP	5 × 5 ml	28-9187-80
HiTrap Desalting	5 × 5 ml	17-1408-01
HiTrap Desalting	100 × 5 ml[1]	11-0003-29
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiPrep 26/10 Desalting	4 × 53 ml	17-5087-02
PD-10 Desalting Column	30	17-0851-01
PD MiniTrap G-25	50	28-9180-07
PD MidiTrap G-25	50	28-9180-08

[1] Special pack size delivered on specific customer order.

Empty lab-scale columns	Pack size	Code No.
Tricorn 5/20 column, 5 mm i.d., max 0.55 ml bed volume or 2.8 cm bed height	1	18-1163-08
Tricorn 5/50 column, 5 mm i.d., max 1.1 ml bed volume or 5.8 cm bed height	1	18-1163-09
Tricorn 10/20 column, 10 mm i.d., max 2.2 ml bed volume or 2.8 cm bed height	1	18-1163-13
Tricorn 10/50 column, 10 mm i.d., max 4.5 ml bed volume or 5.8 cm bed height	1	18-1163-14
Tricorn 10/100 column, 10 mm i.d., max 8.5 ml bed volume or 10.8 cm bed height	1	18-1163-15
XK 16/20 column, 16 mm i.d., max 30 ml bed volume or 15 cm bed height	1	18-8773-01
XK 26/20 column, 26 mm i.d., max 65 ml bed volume or 12.5 cm bed height	1	18-1000-72

Related literature	Code No.
Affinity Chromatography Handbook, Principles and Methods	18-1022-29
Affinity Chromatography Column and Media, Selection Guide	18-1121-86
Recombinant Protein Purification Handbook, Principles and Methods	18-1142-75

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