NeoTrap MBP FPLC Columns

#Cat: NB-19-0079-1mL   Size: 1 ml
#Cat: NB-19-0079-5mL   Size: 5 ml

Introduction

NeoTrap MBP FPLC Columns are designed for simple, one-step and rapid purification of Maltose binding proteins (MBP)-tagged proteins. MBP-tagged recombinant proteins expressed in an insoluble form (inclusion bodies) can be purified in a single step, preserving the activity of the target protein.
Compatible with all common liquid chromatography instruments (including ÄKTA™ FPLC's), peristaltic pumps and syringes.

Specifications

PRODUCT	NeoTrap GST FPLC Columns	
Cat. No.	NB-19-0079-1ml	NB-19-0079-5ml
Column volume	1 ml	5 ml
Resin	Cross-linked agarose	
Bead size	50-150 µm	
Ligand	Dextrin	
Binding capacity	Depends on the type of protein and binding conditions	
Chemical stability	All commonly used buffers, 6M guanidine-HCl, 8 M urea, 0.5 M NaOH (for regeneration and cleaning)	
pH stability	2-13 (short term) and >7 (working range)	
Storage	4-8°C in 20% ethanol	

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Recommended Protocol for Purification:

Buffers needed:

Binding Buffer:	Tris or phosphates at pH>7. Salts, EDTA or DTT can be added to adjust the binding conditions or to stabilize the target proteins.
Elution Buffer:	10 mM to 50 mM maltose in the binding buffer.

Buffers should be sterilized using a filter of 0.22 µm.

The presence of reducing agents, e.g., 5 mM DTT, may decrease yield. Higher ionic strength does not decrease affinity since MBP binds to the resin 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.

INSTRUCTIONS:

1. Column preparation

Connect the NeoTrap column to the pump by removing the end of the column and the top stop plug (save it for storage). Avoid introducing air in the column.

2. Column equilibration

Equilibrate the column with 5 - 10 column volumes of binding buffer until the signal reaches the baseline or becomes stable.

3. Sample application

We recommend filtering the samples though a 0.22 µm filter in order to remove particles before applying it into the column.

4. Column washing

Wash with the binding buffer until the O.D. 280 nm reaches the baseline level again, normally 10-20 column volumes.

5. Purified protein elution

Elute the MBP-tagged protein with 5-10 column volumes of elution buffer and collect the fractions on ice.

6. Regeneration of the column

To elute any reversibly bound material, wash the column with 0.1 – 0.5 M sodium hydroxide.

7. Cleaning-in-place (CIP)

CIP is a procedure that remove strongly bound materials such as lipids, endotoxins and denatured proteins that remain in the column after regeneration. Regular CIP prevents the build up of contaminants in the packed bed and helps to maintain the column performance.
A specific CIP protocol should be developed for each process according to the type of contaminants present. The frequency of CIP depends on the nature of individual applications. The following information works as a general guidance.
The contaminants bound by hydrophobic nature can be removed by the following reagents: 0.5 M NaOH, low percentage non-ionic detergents (e.g. 0.1 – 2%), 30% isopropanol in basic or acidic conditions (e.g. in the presence of acetic acid or phosphoric acid). A combination of the above reagents can be explored as well. In general, the incubation time should be longer (e.g.

from 30 minutes to 2 hours) to ensure full dissociation of the contaminants. Long contact time should be avoided when alcohols are used, as the acrylic column body may be damaged.

8. Sanitation

Sanitation using 0.1-0.5M NaOH with a contact time of 1 hour is recommended.

9. Storage of the column

The resin should be stored in 20% ethanol to prevent microbial growth. Put the top and bottom stop plugs in the column and keep at 4-8 °C. **Do not freeze.**

For reference only

For Research Use Only. Not for Diagnostic or Therapeutic Use.

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