NeoTrap Strep-Tactin® XT 4Flow® FPLC Column

Cat# NB-19-0080-1mL Size: 1 ml

Cat# NB-19-0080-5mL Size: 5 ml

Introduction

Neotrap Strep-Tactin® XT 4Flow® FPLC columns are intended for the purification of Strep-tag®II and Twin-Strep-tag® fusion proteins with HPLC/FPLC devices, such as Äkta® systems. Tagged target proteins can be purified with high purity even from samples with a low target protein concentration, while retaining their biological activity. The elution of the target proteins is performed by a specific competitor, biotin, which releases the tagged target protein from the engineered biotin binding pocket without influencing the target protein's properties. If necessary, biotin can be removed via dialysis, size exclusion chromatography or cross flow ultrafiltration after purification.

Specifications

PRODUCT	NeoTrap Strep-Tactin® XT FPLC Columns	
Cat. No.	NB-19-0080-1ml	NB-19-0080-5ml
Column volume	1 ml	5 ml
Resin	4% agarose, crosslinked	
Bead size	50-150 µm	
Dynamic binding capacity*	14 mg protein/ml resin	
Storage	2-8°C	

*Dynamic binding capacity is protein dependent.

Recommended Protocol for Purification:

BUFFERS NEEDED

Wash Buffer:	100 mM Tris/HCl pH 8.0, 150 mM NaCl,1 mM EDTA. Store at 2-8ºC
Elution Buffer:	100 mM Tris/HCl pH 8.0, 150 mM NaCl, 1 mM EDTA, 50 mM biotin. Store at 2-8ºC
Regeneration Buffer:	3 M $MgCl_2$ Store at 2-8ºC

BIOTIN BLOCKING:

Usually, protein purification and binding capacity of Strep-Tactin® XT 4Flow® resins are not influenced by free biotin, for example in cell culture supernatants, but the co-purification of biotinylated proteins is possible. Biotinylated proteins are only present in the cell in very small amounts, but if a highly pure target protein for analytic applications like mass spectrometry is required, co-purification of biotinylated proteins can be avoided by application of biotin blocking solution containing avidin. Avidin specifically masks biotinylated proteins without influencing the binding properties of the Twin-Strep-tag® or Strep-tag®II.

INSTRUCTIONS:

1. Column preparation

Connect the NeoTrap FPLC 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 NeoTrap FPLC column with 5 CVs (column bed volumes) of Wash Buffer. The **flow rate** should be in the range of **0.5-1 ml/min for 1 ml FPLC columns and 1-3 ml/min for 5 ml FPLC columns**. Monitor the flow through at 280 nm. The baseline should be stable after washing with 5 CVs.

3. Sample application

Centrifuge the sample (18,000 x g, 5 min, 4 °C) to remove any aggregates that may have formed. Apply sample to FPLC column. If the lysate is very viscous and pressure is increased significantly, reduce viscosity of the extract by dilution with Wash Buffer or reduce flow rate.

4. Column washing

Wash with Wash Buffer until A280 is stable. Usually, 5-10 CVs are sufficient to reach the baseline.

5. Purified protein elution

Elute the protein with 5-10 column volumes of Elution Buffer until baseline at A280 is reached. Collect the fractions on ice for future analysis.

6. Regeneration and Storage of the column

Wash with 15 CVs of Regeneration Buffer at a flow rate of 1 ml/min. Immediately remove the Regeneration Buffer by washing with 8 CVs of Wash Buffer.
Put the top and bottom stop plugs in the column and keep at 2-8°C. It can also be stored in 20% ethanol for 6 months without loss in performance. **Do not freeze.**

Neo-Biotech
74, rue des Suisses - 92000 Nanterre