

# NeoTrap Protein A FPLC Columns

Cat# NB-19-0075-1mL size: 1 ml

Cat# NB-19-0075-5mL size: 5 ml

# Introduction

NeoTrap Protein A FPLC Columns are designed for simple, one-step and rapid purification of immunoglobulins from biological fluids and cell culture media. Protein A is trapped by covalent binding, preventing sample loss and allowing the column re-using.

Compatible with all common liquid chromatography instruments (including ÄKTA<sup>™</sup> FPLC's), peristaltic pumps and syringes.

# **Specification**

Protein A consists of a single polypeptide chain which contains five highly homologous antibody-binding domains. The binding site is located on the Fc region of immunoglobulin. Protein A has affinity for IgG from a variety of mammalian species and for some IgA and IgM.

PRODUCT	NeoTrap Protein A FPLC Columns	
Cat. No.	NB-19-0075-1mL	NB-19-0075-5mL
Column volume	1 ml	5 ml
Binding capacity	25 mg	125 mg
Resin	4% crosslinked agarose beads	
Bead size	50-150 μm	
Storage	2-8°C in ethanol 20%	

## **Recommended Protocol for Antibody Purification:**

Binding Buffer:	Sodium phosphate 25 mM
Elution Buffer:	Glycine 0.1 M pH 3
Neutralization Buffer:	Tris Base 1 M pH 9.0
	Elution Buffer:

Buffers should be sterilized using a filter of 0.22  $\mu m.$ 

pH 7.0



#### **INSTRUCTIONS:**

### 1. Column preparation

Purge the pump with Binding Buffer removing all the air.

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.

Wash the column with 10 column volumes of distilled water to eliminate the preservative.

#### 2. Column equilibration

Equilibrate the column with 5 - 10 column volumes of binding buffer.

#### 3. Sample application

It is recommendable to dilute the sample containing the immunoglobulins 1:1 with binding buffer to avoid ionic and pH changes.

All samples should be filtered through a 0.22  $\mu$ m 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 5-10 column volumes.

#### 5. Purified immunoglobulin elution

Elution of the sample is performed at a pH below 3.0. In order to neutralize the eluted fractions, it is recommended the addition of 0.1 ml of neutralization buffer per ml of purified antibody.

## 6. Storage of the column

Put the top and bottom stop plugs in the column and keep at 2-8°C in 20% ethanol. **Do not freeze.** 

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