Data sheet

Fastback Protein A 0.5M NaOH Stable Resin
Super Protein A 0.5M NaOH Stable Resin
Fastback Protein A Big Bead 0.5M NaOH Stable Resin

Neo Biotech's Protein A Sodium Hydroxide Stable Resin range is affinity chromatography media forthe purification of immunoglobulins, such as isolation and purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media. The ligand is specially designed allowing to tolerate higher alkaline concentrations during the cleaning and regeneration step.

The Protein A ligand binds to the Fc region of immunoglobulins. The binding is highly specific sohigh purity can be achieved in a single step.

Fastback Protein A 0.5M NaOH Stable Resin: Suitable for most application from small scale tolarge bioprocessing scale

Super Protein A 0.5M NaOH Stable Resin: Suitable for processing small quantity of antibodies atreduced time

Fastback Protein A Big Bead 0.5M NaOH Stable Resin: Suitable for processing crude or very viscousfeedstocks

This range has been developed and supported for production scale chromatography use.

1. Properties

The Protein A ligand is immobilised to highly porous and highly cross-linked agarose base matrix.Agarose has long been used for chromatographic separations due to its excellent hydrophilic andlow non-specific-binding nature. The particles have an open pore structure with excellent mass transfer properties to large protein molecules. The medium shows high mechanical rigidity, so it can be operated at moderate to high flow velocities with moderate pressure drops.

Neo Biotech offers both loose medium and pre-packed ready-to-use disposable columns.

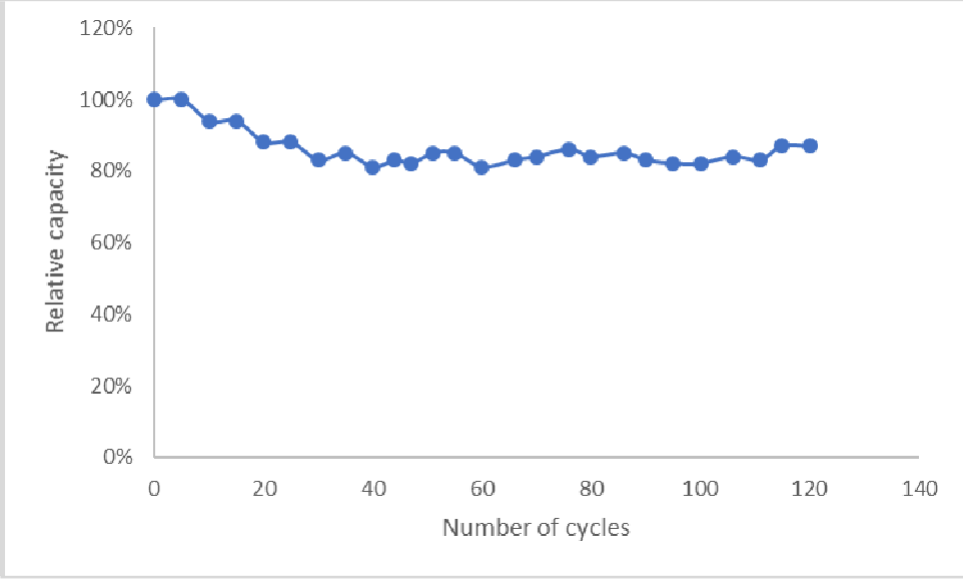

Figure-1: Fastback Protein A alkaline stability test at 0.5 M NaOH: the resin was cleaned with 0.5 M NaOH for 15 mins after each binding / elution cycle with hIgG

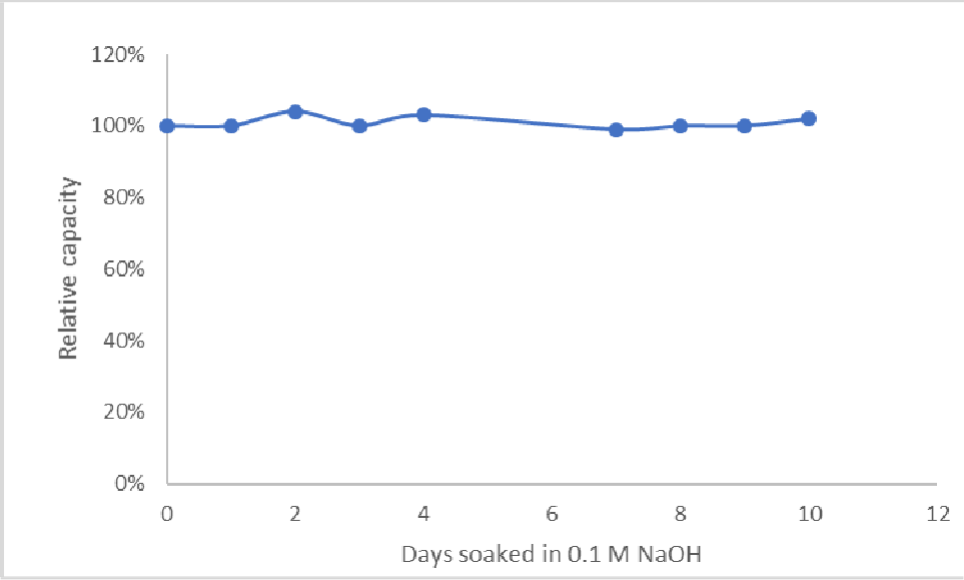

Figure-2: Fastback Protein A alkaline stability test at 0.1 M NaOH: the resin was soaked in 0.1M NaOH and a sample was taken each day to test its binding capacity to hIgG

Table 1: Characteristics of Protein A Media and Column:

Matrix	Highly cross-linked Agarose
Particle size	50 - 150 μm (Fastback Protein A)20 - 50 μm (Super Protein A) 150 - 350 μm (Fastback Protein A Big Bead)
Binding capacity	>30 mg/ml human IgG/ml
Column material	Polypropylene (end-caps, stop plugs), acrylic or polypropylene (column body), polypropylene or polyethylene (frit or meshes),NBR O-rings
Operational pressure	Up to 3 bar (42 psi)
pH stability	2-10 (short term) and 3-9 (long term)
Working temperature	+4^{O}C to +30^{O}C
Chemical stability	Compatible with most commonly used reagents for antibody purifications
Sanitisation	0.5 M NaOH for 15 minutes
Storage	20% ethanol at +4^{O}C - +8^{O}C

2. General operations

This Protein A range can be used in batch stirred tank, gravity flow or packed bed operations.

The pre-packed resin is stored in 20% ethanol on delivery. It can be directly connected to a suitable chromatography system such as AKTA. Be sure that it is air bubble free. Normally, the endwith the product label should be connected as the top inlet. If there is no label difference between those two ends, the column can be connected either way.

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The resin or column should be equilibrated with at least 5 – 10 column volumes of the equilibrationbuffer until the pH and conductivity signals become stable, before a sample is loaded.

The running pressure shall not exceed 3 bars during the operation.

After each application, seal the column ends and store the column properly if re-use is expected.

3. Binding

These Protein A resins bind IgG from most species at neutral pH (e.g. pH 7 to 7.4) and physiological ionic strength (e.g. phosphate saline buffer). The static binding capacity depends onthe source of the particular immunoglobulin. For a column operation, the dynamic binding capacityis determined by a few factors such as flow rate (residence time), sample concentration and binding buffer.

4. Elution

The bound immunoglobulin is normally eluted by reduced pH, such as about pH 3.0. The generalelution buffer includes 0.1M glycine pH 3.0 or 0.1M citric acid pH 3.0. For very strongly bound molecules, the pH may reduce to between 2 to 3.

For acid labile proteins, the eluted fractions can be quickly neutralized by adding (or with pre-added) 1M Tris/HCl, pH 9.0 (10% to 20% v/v).

5. Regeneration

After the elution, wash the medium with 2 – 3 volumes of the elution buffer following with 3 – 5volumes of the equilibration buffer.

6. Cleaning-in-place (CIP)

In some applications, substances such as denatured proteins or lipids stay in the column after theregeneration step. The following cleaning procedure could be carried out.

To remove precipitated or denatured materials, wash the column with 2 column volumes of 6 M guanidine hydrochloride followed immediately with at least 5 column volumes of the binding buffer.To remove the bound hydrophobic components, wash the column with 1 column volume of a non- ionic detergent e.g. 0.1% Triton™ X-100 at 37ºC followed immediately with at least 5 column volumes of the binding buffer.

Note: washing with concentrated alcohol is not recommended if the column body is made of acrylic material.

7. Sanitization

Introduce 0.5 M NaOH to the column and keep re-circulating the alkaline solution in the column for15 minutes. Quickly displace the alkaline solution with the equilibration buffer until the column is equilibrated.

8. Storage

Store the loose medium or the pre-packed column in the presence of 20% ethanol at 4-8ºC. Neverfreeze the medium or the column.

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9. Further information

Visit www.neobiotech.com for further information or contact the technical team or salesrepresentatives.

10. Ordering information

Product Description	Pack Size	Product Code
Super Recombinant Protein A, 0.5M NaOH Stable Resin	5 ml 25 ml 100 ml 250 ml 500ml 1 L 5 L 10 L	NB-45-0223-5 NB-45-0223-25 NB-45-0223-100 NB-45-0223-250 NB-45-0223-500 NB-45-0223-1L NB-45-0223-5L NB-45-0223-10L
Fastback Recombinant Protein A, 0.5M NaOH Stable Resin	5 ml 25 ml 100 ml 250 ml 500ml 1 L 5 L 10 L	NB-45-0245-5 NB-45-0245-25 NB-45-0245-100 NB-45-0245-250 NB-45-0245-500 NB-45-0245-1L NB-45-0245-5L NB-45-0245-10L
Fastback Recombinant Protein A Big Bead, 0.5M NaOH Stable Resin	5 ml 25 ml 100 ml 250 ml 500ml 1 L 5 L 10 L	NB-45-0244-5 NB-45-0244-25 NB-45-0244-100 NB-45-0244-250 NB-45-0244-500 NB-45-0244-1L NB-45-0244-5L NB-45-0244-10L

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