iba
Solutions
For Life Sciences

Protein Production &
Assays

Protein A Agarose

Instruction manual

Last date of revision
November 2018

Version PR75-0001

fisher biotec australia
MORE THAN 20 YEARS IN BUSINESS Established 1997
Australian distributors:
Fisher Biotec Australia
free call: 1800 066 077
email: info@fisherbiotec.com
web: www.fisherbiotec.com

For research use only

Content

1 Introduction

1.1 General information on Protein A Agarose

Protein A Agarose is an affinity medium for capturing antibodies from large sample volumes. As the name indicates the resin is based on an Agarose matrix with a recombinant Protein A ligand.

Protein A is a surface protein originally found in the cell wall of the bacteria *Staphylococcus aureus*. It has found use in biochemical research because of its ability to bind immunoglobulins. It is composed of five homologous Ig-binding domains that fold into a three-helix bundle. Each domain is able to bind proteins from many mammalian species, most notably IgGs.

The recombinant Protein A ligand is expressed in *E. coli* and is free of components of mammalian origin. The specificity of binding to the Fc region of IgG is similar to that of native Protein A giving a high purification factor in a single step.

1.2 Protein A Agarose capacity and stability

Figure 1 and 2 show the relation between dynamic binding capacity and residence time for Protein A Agarose.

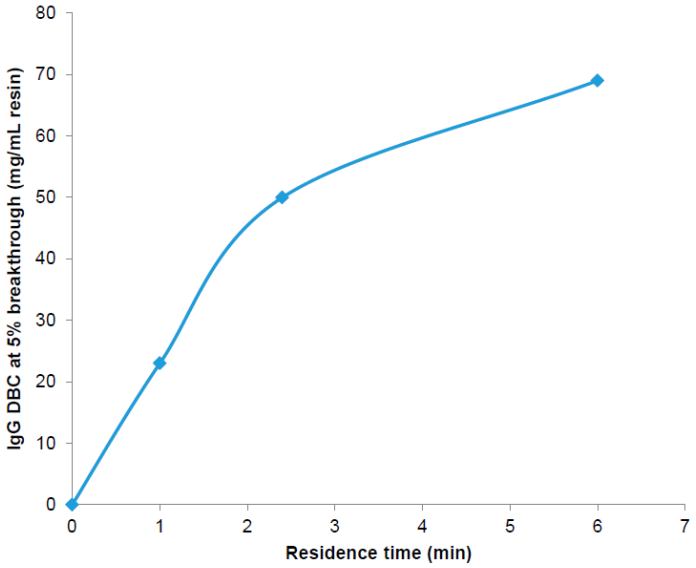


Fig 1. Relation between dynamic binding capacity at 5% breakthrough and residence time for Protein A Agarose for a purified monoclonal antibody

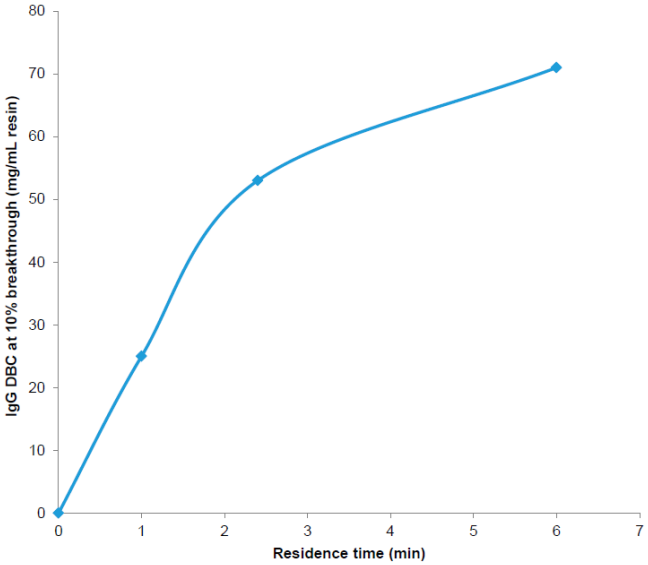


Fig 2. Relation between dynamic binding capacity at 10% breakthrough and residence time for Protein A Agarose for a purified monoclonal antibody

Figure 3 shows stability in alkaline conditions of Protein A Agarose in terms of dynamic binding capacity.

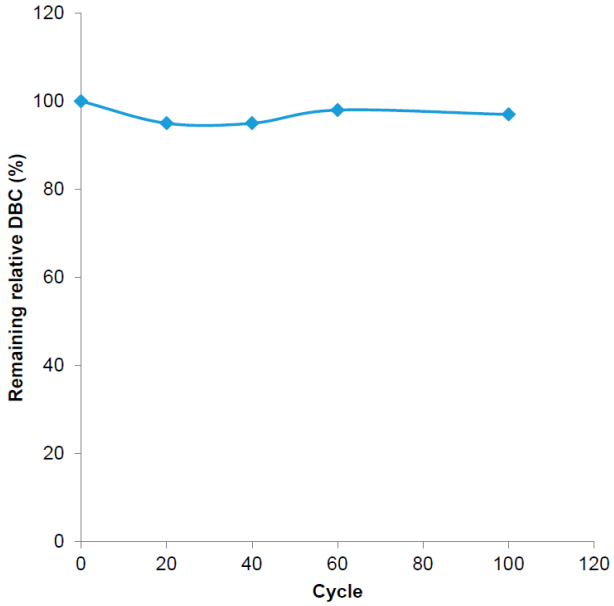


Fig 3.: Dynamic binding capacity for Protein A Agarose after CIP with 0.1 or 0.5 M NaOH, 15 min contact time, for 0–100 cycles.

The dynamic binding capacity (DBC, 10% breakthrough) for Protein A Agarose was measured after 20, 40, 60 and 100 cycles.

2 Antibody purification using gravity columns

2.1 Purification Protocol

Recommended Buffers/Solutions	Concentration of ingredients	Notes
Storage buffer	PBS 1 mM EDTA 0.01 % NaN_3 pH = 7.2 – 7.4	
Buffer A (Binding and wash buffer)	PBS pH = 7.2 – 7.4	
Buffer B (Elution buffer)	0.1 M Glycin/HCl pH = 2.7	
Buffer N (Neutralization buffer)	1 M Tris/HCl pH = 8.5	

Important notes	• The sample should be in the same buffer as the composition of the binding buffer. • This can be done by dilution with binding buffer or by re-buffering. • Pass sample through a 0.45 µm or a 0.2 µm filter in order to remove any precipitates. • Alternatively spin down sample right before usage. • All buffers should be degassed.

Protocol	**1. Equilibrate column with 3 column bed volumes (CVs) of buffer A (optional).** **2. Slowly load sample onto the column.** Note: Try to avoid overloading the column in order to prevent sample loss. **3. Wash the column with 5-10 CVs of buffer A.** Efficiency of washing can be monitored via OD (280 nm) of flow through. **4. Elute first elution fraction with 0.5 CVs of buffer B.** **5. Elute 5 consecutive elution fractions using 1 CV of buffer B for each**

Protocol (continued)	**fraction.** **6. Add 1/10th of elution volume of buffer N to each fraction and vortex gently.** Note: Try to avoid foam formation. **7. You can determine the antibody concentration of the elution fractions by measuring the OD at 280 nm. An OD_{280} of 1.38 is equivalent to 1 mg/mL antibody.** **8. If desired, fractions can be pooled and the final concentration can be determined again.**

3 Antibody purification using FPLC

3.1 Purification Protocol

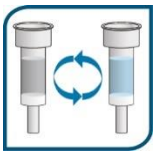
Recommended Buffers/Solutions	Concentration of ingredients	Notes
Storage buffer	PBS 1 mM EDTA 0.01 % NaN_3 pH = 7.2 – 7.4	
Buffer A (Binding and wash buffer)	PBS pH = 7.2 – 7.4	
Buffer B (Elution buffer)	0.1 M Glycin/HCl pH = 2.7	
Buffer N (Neutralization buffer)	1 M Tris/HCl pH = 8.5	

Important notes

- The sample should be in the same buffer as the composition of the binding buffer.
- This can be done by dilution with binding buffer or by re-buffering.
- Pass sample through a 0.45 µm or a 0.2 µm filter in order to remove any precipitates.
- Alternatively spin down sample right before usage.
- All buffers should be degassed.

Protocol

1. **Set FPLC system flow rate to 0.5 – 1 mL/min for a 1 mL column and 1 – 3 mL/min for a 5 mL cartridge.**
2. **Equilibrate the cartridge with 5 CVs of buffer A (optional).**
3. **Load sample.**
4. **Wash with 5 – 10 CVs of buffer A.**
5. **Elute with 6 CVs of buffer B.**
6. **Add 1/10th of elution volume of buffer N to each fraction vial and vortex gently.**
   Note: Try to avoid foam formation

	7. If desired fractions can be pooled.	

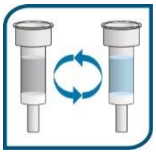
3.2 Regeneration Protocol

Recommended Buffers/Solutions	Concentration of ingredients	Notes
Buffer A (Binding and wash buffer)	PBS pH = 7.2 – 7.4	
Buffer B (Elution buffer)	0.1M Glycin/HCl pH = 2.7	

Important notes	• All buffers should be degassed.

Protocol	**1. Regenerate cartridge using 5 CVs of buffer B.** **2. Wash the column with at least 5 CVs of buffer A or until the column has reached the same pH as buffer A.**

4 Cleaning-in-place (CIP)



CIP is the removal of tightly bound precipitated or denatured substances from the resin. If such contaminants remain in the system, they may affect the chromatographic properties of the material, reduce the binding capacity and, potentially, come off in subsequent runs.

Recommended Buffers/Solutions	Concentration of ingredients	Notes
CIP solution	0.1 M – 0.5 M NaOH	
Storage buffer	PBS 1 mM EDTA 0.01 % NaN_3 pH = 7.2 – 7.4	

Important notes

- All buffers should be degassed.

Protocol

1. **Equilibrate column/cartridge with 3 CVs of H_2O.**
2. **Rinse column/cartridge with at least 3 CVs of 0.1 – 0.5 M NaOH.**
   Note: Contact time should be at least 15 minutes.
3. **Rinse column/cartridge with storage buffer until pH 7.2 – 7.4 is reached.**

5 Trouble Shooting

5.1 General issues

pH of the sample is not correct	The pH should be ≥ 7.2
Low yield	Reduced flow rates may increase yields of the target protein (optimize residence time)
Unstable pressure curve during sample application	• Remove air bubbles that might have been trapped in the sample pump • Degas the sample using a vacuum degasser
High back pressure during the run	• The column may be clogged • Perform CIP
Sample too viscous	• Dilute sample with PBS buffer • Reduce flow rate during sample loading
Column bed compressed	• Check sample preparation. If sample is too viscous, back pressure will be high. Dilute sample with PBS or reduce flow rate • Perform CIP to clean Protein A cartridge
Microbial growth in column	• Perform CIP with 0.1-0.5 M NaOH • Store material in presence of 20 % ethanol or PBS/1 mM EDTA/0.01 % Na_3 when not in use. • Always filter samples and buffers

6 Notes

IBA GmbH

Solutions
For Life Sciences

Australian distributors:
Fisher Biotec Australia
free call: 1800 066 077
email: info@fisherbiotec.com
web: www.fisherbiotec.com