Protein Production & Assays

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Protocol

ANTIBODY PURIFICATION WITH PROTEIN A AGAROSE

1 DESCRIPTION

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 coupled 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 can 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.

2 INITIAL PREPARATIONS

The composition of the required buffers, which are not provided by IBA Lifesciences, is listed below. Prior to application, the buffers should be degassed.

Storage Buffer	PBS, 1 mM EDTA, 0.01% NaN_3, pH 7.2-7.4
Buffer A	PBS, pH 7.2-7.4 (binding and wash buffer)
Buffer B	0.1 M Glycin/HCl, pH 2.7 (elution buffer)
Buffer N	1 M Tris/HCl, pH 8.5 (neutralization buffer)
CIP solution	0.1-0.5 M NaOH

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. Please note that the sample should be pass through a 0.45 µm or a 0.2 µm filter prior to purificaiton in order to remove any precipitates.

3 PROTOCOL

3.1. Antibody purification using Protein A Agarose gravity flow columns

3.1.1 Optional: equilibrate the column with 3 column bed volumes (CVs) Buffer A.
3.1.2 Slowly load sample onto the column. Avoid overloading of the column to prevent sample loss.
3.1.3 Wash the column with 5-10 CVs Buffer A. Washing efficiency can be monitored via OD_{280}. Collect the wash fractions for SDS-PAGE analysis.
3.1.4 For the first elution fraction apply 0.5 CV Buffer B and then apply five times 1 CV Buffer B. Collect all six fractions separately for SDS-PAGE analysis.
3.1.5 To neutralize the pH of the eluate, add one-tenth of the elution volume of Buffer N to each elution fraction and vortex gently. Avoid the formation of foam.

3.1.6 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. If desired, fractions can be pooled, and the final concentration can be determined again.
3.1.7 Regenerate the cartridge with 5 CVs of Buffer B.
3.1.8 Wash the column with at least 5 CVs of Buffer A or until the column has reached the same pH as Buffer A.

3.2 Antibody purification using Protein A Agarose cartridges

3.2.1 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.
3.2.2 Optional: equilibrate the column with 5 column bed volumes (CVs) Buffer A.
3.2.3 Load the sample.
3.2.4 Wash with 5-10 CVs Buffer A. Washing efficiency can be monitored via OD_{280}. Collect the wash fractions for SDS-PAGE analysis.
3.2.5 Elute with 6 CV Buffer B.
3.2.6 To neutralize the pH of the eluate, add one-tenth of the elution volume of Buffer N to each elution fraction and vortex gently. Avoid the formation of foam.
3.2.7 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.
3.2.8 Regenerate the cartridge with 5 CVs of Buffer B.
3.2.9 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 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.

4.1 Equilibrate the column/cartridge with 3 CV H_2O.
4.2 Rinse column/cartridge with at least 3 CV CIP solution. Contact time should be at least 15 minutes.
4.3 Rinse column/cartridge with storage buffer until pH 7.2-7.4 is reached.

5 TROUBLESHOOTING

Low binding	The pH of the sample is not correct. It 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 could 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 the cartridge/column.
Microbial growth in column	Perform CIP. Store material in presence of 20% ethanol or Storage Buffer. Always filter samples and buffers

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If you have any questions, please contact

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We are here to help!