

Batch and spin cup methods for affinity purification of proteins

TR0004.2

Introduction

Affinity purification is a technique enabling isolation of a molecule of interest based on its specific binding affinity to a particular ligand that has been immobilized on a solid support (usually beaded agarose resin). Sample containing the molecule of interest is incubated with the immobilized ligand resin in buffer conditions that facilitate binding. After non-bound components of the sample are washed away from the resin with additional buffer, the molecule of interest is recovered (eluted) from the resin using an altered buffer condition that disrupts the ligand-molecule binding.

For purifications involving protein:protein affinities, binding buffers with physiological pH and ionic strength are generally appropriate. Phosphate-buffered saline (PBS, Product No. 28372) is often used for this purpose. Buffers such as 0.1 M glycine•HCl (pH 2.5) or IgG Elution Buffer (Product No. 21004) are usually appropriate for elution. For more information on binding and elution buffers, see Tech Tip #27: Optimize elution conditions for immunoaffinity purification.

Traditionally, affinity purification is performed in column format, where the sample is applied and eluted by gravity flow through a packed resin bed of one to several milliliters. While a column format is suitable for applications that involve large quantities of immobilized ligand and purified product (e.g., > 1 mg of protein or peptide), a smaller batch format is more suitable when the sample volume is small and only microgram amounts of purified product are required for subsequent analysis (e.g., by gel electrophoresis and Western blotting detection).

Batch method purification can be performed at any scale. However, it is most commonly reserved for microcentrifuge tube scale purifications involving 10-200 μ l of resin. In batch method purification, wash and elution fractions are separated from the resin after centrifuging to pellet the resin beads. The liquid cannot be removed completely because some of it is contained within the volume of porous bead pellet. Consequently, a portion of each fraction about equal to the volume of resin used is left behind in the pellet, making washes and elution somewhat inefficient.

The spin cup purification method provides improved efficiency of wash and elution steps relative to the batch method. Centrifugation separates the liquid fraction by withdrawing it thoroughly from the resin, which is retained within the spin cup apparatus. Spin cup purification is most appropriate when 50-300 μ l of immobilized ligand resin is used.

Example small-scale batch and spin cup protocols for affinity purification of IgG (antibody) molecules using Protein A agarose beads (Immobilized Protein A, Product No. 20333) are given below. For other affinity systems, substitute the appropriate affinity resin and binding and elution buffers.

Materials Required

- Microcentrifuge tube(s): Handee™ Microcentrifuge Tubes (Product No. 69720)
- Spin cups (needed for spin cup method only): Handee™ Spin Cups – Cellulose Acetate Filter (Product No. 69702)
- Immobilized Protein A (Product No. 20333)
- Binding Buffer: Phosphate-buffered saline (Product No. 28372)
- Elution Buffer: IgG Elution Buffer (Product No. 21004) or 0.1 M glycine•HCl (pH 2.5-2.8)
- Pipetters and wide-bore or end-cut pipette tips

Procedure for Batch Method Purification

Note: This example uses 50 µl of resin. For different amounts of resin, adjust buffer volumes proportionately. Use a volume of sample appropriate to the binding capacity of the resin.

- Using a wide-bore tip, pipette 100 µl of Immobilized Protein A resin slurry into a microcentrifuge tube.
Note: Because the beaded agarose resin is supplied as a 50% slurry, pipetting 100 µl results in 50 µl of settled resin.
- Cap the tube and centrifuge 15 seconds at high speed in a microcentrifuge (i.e., about 10,000 x g).
- Carefully remove the supernatant by pipette and discard it.
- Add 50 µl of Binding Buffer, cap the tube and resuspend the resin by flicking or gently vortexing the tube.
- Centrifuge 15 seconds at high speed; carefully remove the supernatant by pipette and discard it.
- Repeat steps 4 and 5 twice for a total of three washes.
- Add 50 µl of serum (diluted 1:1 with Binding Buffer), cap tube and resuspend the resin by flicking or gently vortexing.
- Incubate sample-resin mixture at room temperature for 15-90 minutes. Periodically resuspend the resin.
- Centrifuge 15 seconds at high speed; carefully remove the supernatant by pipette.
Note: Save the supernatant; it contains the non-bound proteins. Later analysis of this fraction for the target antibody can help in assessing success of the affinity purification and if the binding capacity of the resin was exceeded by the sample.
- Add 50 µl of Binding Buffer, cap the tube and resuspend the resin by flicking or gently vortexing.
- Centrifuge 15 seconds at high speed; carefully remove the supernatant by pipette.
- Repeat steps 10 and 11 four times for a minimum of five washes. Additional washes using 1 M NaCl may help to remove nonspecifically bound material.
- Add 50 µl of Elution Buffer, cap the tube and resuspend the resin by flicking or gently vortexing.
- Incubate for 5 minutes, then centrifuge 15 seconds at high speed. Transfer the supernatant (contains the eluted IgG) to a clean tube.
Note: Elution fractions obtained using 0.1 M glycine•HCl (pH 2.5) or IgG Elution Buffer (Product No. 21004) can be neutralized by adding 3-5 µl of 1 M Tris or 1 M phosphate buffer, pH 8.0.
- Repeat steps 13 and 14 four times for a minimum of five separate elution fractions. Fractions 1-3 will most likely contain all the eluted IgG. The eluted IgG exchanged into a suitable storage buffer using a dialysis unit or desalting column (see Related Pierce Products).

Procedure for Spin Cup Method Purification

Note: This example uses 200 µl of resin. For different amounts of resin, adjust buffer volumes proportionately. Use a volume of sample appropriate to the binding capacity of the resin.

- Using a wide-bore tip, pipette 400 µl of Immobilized Protein A resin slurry in a spin cup column.
Note: Because the beaded agarose resin is supplied as a 50% slurry, pipetting 400 µl results in 200 µl of settled resin.
- Place spin cup in a microcentrifuge collection tube, cap it and centrifuge the assembly for 1 minute at 1,000 × g (i.e., about 3,000 rpm in a microcentrifuge).
- Discard liquid from the collection tube.
- Add 150 µl of Binding Buffer to the spin cup, cap the unit and resuspend the resin.
- Centrifuge 1 minute at 1,000 × g; discard liquid from the collection tube.
- Repeat steps 4 and 5 twice for a total of three washes.
- Add serum sample (diluted 1:1 with Binding Buffer) to the resin in the spin cup. Cap unit and resuspend the resin.
Note: For dilute IgG samples such as culture supernatants, incubate a large volume of sample with the resin in a separate tube that is large enough to hold the entire sample-resin volume. After incubation, transfer the resin pellet back to the spin cup, and proceed to step 10. Save the sample solution (see note for step 9).

8. Incubate at room temperature for 15-90 minutes. Periodically resuspend the resin.
9. Centrifuge 1 minute at $1,000 \times g$; do not discard the collected solution.
Note: Save the solution; it contains the unbound proteins. Later analysis of this fraction for the target antibody can help in assessing success of the affinity purification and if the binding capacity of the resin was exceeded by the sample.
10. Add 150 μ l of Binding Buffer, cap the tube and resuspend the resin by flicking or gently vortexing the tube.
11. Centrifuge 1 minute at $1,000 \times g$; discard liquid in the collection tube or transfer it into a different tube.
12. Repeat steps 10 and 11 four times for a minimum of five washes. Additional washes using 1 M NaCl can help to remove nonspecifically bound material.
13. Add 150 μ l of IgG Elution Buffer, resuspend resin and incubate with gentle mixing for five minutes. Transfer the spin cup column to a clean collection tube.
14. Centrifuge 1 minute at $1,000 \times g$ to elute the bound IgG. Transfer liquid from the collection tube to a clean vial.
Note: Elution fractions obtained using 0.1 M glycine•HCl (pH 2.5) or IgG Elution Buffer (Product No. 21004) can be neutralized by adding 3-5 μ l of 1 M Tris or phosphate buffer, pH 8.0.
15. Repeat steps 13 and 14 four times for a minimum of five separate elution fractions. Fractions 1-3 will most likely contain all the eluted IgG. The eluted IgG exchanged into a suitable storage buffer using a dialysis unit or desalting column (see Related Pierce Products).

Related Pierce Products

20398	Immobilized Protein G , 2 ml
20421	Immobilized Protein A/G , 3 ml
20510	Immobilized Protein L , 2 ml
21027	Gentle Ag/Ab Elution Buffer , 500 ml
69570	Slide-A-Lyzer[®] MINI Dialysis Unit , 50/pkg, 10 K MWCO, for dialysis of 10-100 μ l samples
66385	Slide-A-Lyzer[®] Dialysis Cassette Kit , includes 10 cassettes and accessories, 10 K MWCO, for dialysis of 0.1-0.5 ml samples
89882	Zeba[™] Desalt Spin Columns , 25/pkg, 5 K MWCO, for 30-130 μ l samples
89889	Zeba[™] Desalt Spin Columns , 5/pkg, 5 K MWCO, for 200-700 μ l samples
22230	Immobilized Affinity Ligand Techniques , Greg T. Hermanson, A. Krishna Mallia, and Paul K. Smith, Published by Academic Press, Inc., 1992, 450 pages; comb-bound.

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