TECHNICAL RESOURCE

Batch and spin cup methods for affinity purification of proteins

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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 gel). Sample containing the molecule of interest is incubated with the immobilized ligand gel in buffer conditions that facilitate binding. After unbound components of the sample are washed away from the gel with additional buffer, the molecule of interest is recovered (eluted) from the immobilized ligand gel 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 ImmunoPure® IgG Elution Buffer (Product No. 21004) are usually appropriate for elution.

Traditionally, affinity purification is performed in column format, where the sample is applied and eluted by gravity flow through a packed gel 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 µl of gel. In batch method purification, wash and elution fractions are separated from the gel after centrifuging to pellet the gel. The liquid cannot be removed completely because some of it is contained within the volume of gel. Consequently, a portion of each fraction equal to at least the volume of gel used is left behind in the pelleted gel, 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 from the gel by withdrawing it thoroughly from the gel, which is retained within the spin cup apparatus. Spin cup purification is most appropriate when 50-300 µl of immobilized ligand gel is used.

Example batch and spin cup protocols for affinity purification of IgG (antibody) molecules using immobilized Protein A agarose (Product No. 20333) are given below.

Materials Required

- Microcentrifuge tube(s): Handee™ Microcentrifuge Tubes (Product No. 69715)
- Spin cups (needed for the spin cup column method only): Handee™ Spin Cup Columns (Product No. 69700)
- Immobilized Protein A: ImmunoPure® Immobilized Protein A (Product No. 20333)
- Binding Buffer: ImmunoPure® (A) IgG Binding Buffer (Product No. 21001)
- Elution Buffer: ImmunoPure® IgG Elution Buffer (Product No. 21004)

Procedure for Batch Method Purification

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

1. Pipette 100 µl of Immobilized Protein A gel slurry into a microcentrifuge tube.

   Note: Since the gel is supplied as a 50% slurry, pipetting 100 µl results in 50 µl of settled gel.

2. Cap the tube and centrifuge 15 seconds at high speed in a microcentrifuge (i.e., about 10,000 x *g*).
3. Carefully remove the supernatant by pipette and discard it.
4. Add 50 µl of Binding Buffer, cap the tube and resuspend the gel by flicking or gently vortexing the tube.
5. Centrifuge 15 seconds at high speed; carefully remove the supernatant by pipette and discard it.
6. Repeat steps 4 and 5 twice for a total of three washes.
7. Add 50 µl of serum (diluted 1:1 with Binding Buffer), cap the tube and resuspend the gel by flicking or gently vortexing the tube.
8. Incubate sample with gel at room temperature for 15-90 minutes. Periodically resuspend the gel.
9. Centrifuge 15 seconds at high speed; carefully remove the supernatant by pipette.

   Note: Save the supernatant; 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 gel was exceeded by the sample.

10. Add 50 µl of binding buffer, cap the tube and resuspend the gel by flicking or gently vortexing the tube.
11. Centrifuge 15 seconds at high speed; carefully remove the supernatant by pipette.
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 50 µl of Elution Buffer, cap the tube and resuspend the gel by flicking or gently vortexing the tube.
14. Incubate for 5 minutes, then centrifuge 15 seconds at high speed. Transfer supernatant (contains the eluted IgG) to a clean vial.

    Note: Elution fractions obtained using 0.1 M glycine•HCl (pH 2.5) or ImmunoPure® IgG Elution Buffer (Product No. 21004) can be neutralized by adding 3-5 µl of 1 M Tris or phosphate buffer, pH 7.5.

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 can be dialyzed against a suitable storage buffer using Slide-A-Lyzer® MINI Dialysis Units or Slide-A-Lyzer® Dialysis Cassettes (see Related Pierce Products).

Procedure for Spin Cup Method Purification

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

1. Place 400 µl of Immobilized Protein A gel slurry in a spin cup column.

   Note: Since the gel is supplied as a 50% slurry, pipetting 400 µl results in 200 µl of settled gel.

2. Place spin cup in a microcentrifuge collection tube, cap it and centrifuge the assembly for 1 minute at 1,000 x *g* (i.e., about 3,000 rpm in a microcentrifuge).
3. Discard liquid from the collection tube.
4. Add 150 µl of Binding Buffer to the spin cup, cap the unit and resuspend the gel.
5. Centrifuge 1 minute at 1,000 x *g*; discard liquid from the collection tube.
6. Repeat steps 3 and 4 twice for a total of three washes.

7. Add an appropriate volume of serum (diluted 1:1 with Binding Buffer) to the gel in the spin cup. Cap unit and resuspend the gel.

   Note: For dilute IgG samples such as culture supernatants, incubate a large volume of sample with the gel in a separate tube that is large enough to hold the entire sample/gel volume. After incubation, transfer the gel 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 gel.

9. Centrifuge 1 minute at 1,000 x *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 gel was exceeded by the sample.

10. Add 150 µl of Binding Buffer, cap the tube and resuspend the gel by flicking or gently vortexing the tube.

11. Centrifuge 1 minute at 1,000 x *g*; discard liquid in the collection tube.

12. Repeat steps 8 and 9 four times for a minimum of five washes. Additional washes using 1 M NaCl can help to remove nonspecifically bound material.

13. Add 150 µl of ImmunoPure® IgG Elution Buffer, resuspend gel 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 x *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 ImmunoPure® IgG Elution Buffer (Product No. 21004) can be neutralized by adding 3-5 µl of 1 M Tris or phosphate buffer, pH 7.5.

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 can be dialyzed against a suitable storage buffer using Slide-A-Lyzer® MINI Dialysis Units or Slide-A-Lyzer® Dialysis Cassettes (see Related Pierce Products).

Related Pierce Products

20398	**ImmunoPure® Immobilized Protein G,** 2 ml
21007	**ImmunoPure® (A) IgG Binding Buffer,** 3.75 liters
21009	**ImmunoPure® IgG Elution Buffer,** 3.75 liters
21011	**ImmunoPure® (G) IgG Binding Buffer,** 3.75 liters
21020	**ImmunoPure® Gentle Ag/Ab Binding Buffer,** 1 liter
21027	**ImmunoPure®Gentle Ag/Ab Elution Buffer,** 500 ml
69570	**Slide-A-Lyzer® MINI Dialysis Unit,** 50/pkg, 3.5 K MWCO, for dialysis of 10-100 µl samples
69552	**Slide-A-Lyzer® Dialysis Cassette Kit,** includes 10 cassettes and accessories, 3.5 K MWCO, for dialysis of 0.5-3 ml 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.