Protein A (or A/G) Antibody Purification Kit

Catalog No.: BRC0017

Product Description

EnkiBeads™ Protein A (or A/G) Antibody Purification Kit utilizes EnkiLife's proprietary protein coupling technology to covalently immobilize Protein A (or A/G) onto NHS-activated superparamagnetic microspheres. Compared to similar products on the international market, this kit offers higher antibody binding capacity and lower non-specific protein adsorption, with more uniform elution conditions. A single step purification yields >90% pure antibody from serum samples.

This product features nanoscale magnetic microspheres with an ultra-large specific surface area, significantly reducing antibody binding time. Skilled users can complete the antibody binding process within 15 minutes and the entire purification workflow within 30 minutes. The kit includes pre-optimized buffer formulations that provide optimal reaction conditions for antibody purification, ensuring enhanced protocol stability and reproducibility.

The product is reusable and suitable for antibody purification from plasma, ascites, tissue culture supernatant, and other sample types. It can also be used for antibody immobilization and related research applications. Users should select the appropriate bead type (Protein A or Protein A/G) based on the species and isotype of the target antibody. Refer to Appendix 1 for the affinity comparison between Protein A/G beads and various antibodies.

Kit Components

No.	Reagent Name	Size
Reagent 1	MagProtein A (or A/G) Beads	1 mL
Reagent 2	Antibody Binding Buffer	100 mL
Reagent 3	Antibody Elution Buffer I (pH 4.5)	50 mL
Reagent 4	Antibody Elution Buffer II (pH 2.0)	50 mL
Reagent 5	Antibody Neutralization Buffer	10 mL
Reagent 6	Bead Washing Buffer	10 mL
Reagent 7	Bead Storage Buffer	10 mL
Reagent 8	Bead Regeneration Buffer	10 mL

Storage Conditions and Shelf Life

Storage Conditions: Store at 2–8°C. DO NOT FREEZE.
Shelf Life: 2 years
Binding Capacity:

- **Protein A:** 1.4–1.6 mg Human IgG/mL beads

- **Protein A/G:** 1.8–2.0 mg Human IgG/mL beads

Standard Operating Protocol

Different magnetic beads have varying antibody binding capacities. Before purification, estimate the antibody concentration in your sample (serum typically contains 2–8 mg/mL; cell culture supernatant varies by expression level). Based on the bead's binding capacity (see product specifications), calculate the required bead volume. Optimal bead usage is 80–100% of the sample's antibody content. Using too many or too few beads will affect purification efficiency.

The following protocol uses 100 µL of Protein A beads as an example. Adjust all reagent volumes proportionally for your specific application.

Step 1. Sample Preparation

Transfer sample containing ~0.1–0.15 mg antibody to a new 1.5 mL microcentrifuge tube. Add Antibody Binding Buffer to a final volume of 500 µL (if sample volume >500 µL, no additional buffer is needed). Mix well.

Step 2. Bead Pre-treatment

Vortex the bead suspension vigorously for 30 seconds to fully resuspend. Transfer 100 µL of beads to a new 1.5 mL tube. Separate magnetically (place tube on magnetic stand) until solution clears. Remove supernatant. Remove tube from magnet. Wash beads twice with Antibody Binding Buffer, remove supernatant after each wash. Beads are now ready for use.

Step 3. Antibody Binding

Add the prepared sample from Step 1 to the pre-washed beads. Vortex to mix. Incubate at room temperature (25°C) with gentle rotation or end-over-end mixing for 15 minutes to ensure complete binding. Magnetically separate and remove supernatant.

Step 4. Bead Washing

Add 1 mL of Antibody Binding Buffer, resuspend beads, magnetically separate, and remove supernatant. Repeat this wash step 3 times total.

Step 5A. Antibody Elution (Method 1: Mild Acidic, High Salt)

1. **Elution:** Add 0.5–1.0 mL of Antibody Elution Buffer I (pH 4.5) to washed beads. Resuspend quickly by pipetting or vortexing. Incubate with gentle rotation for 10 minutes at room temperature. Magnetically separate and collect supernatant in a new tube.
2. **Dialysis:** Elution Buffer I contains high salt. The eluted antibody cannot be directly analyzed by SDS-PAGE but can be quantified using this buffer as blank. Perform immediate dialysis against a neutral, low-salt buffer to maintain antibody activity and stability.

Step 5B. Antibody Elution (Method 2: Low pH)

1. **Elution:** Add 0.5–1.0 mL of Antibody Elution Buffer II (pH 2.0) to washed beads. Resuspend quickly. Incubate with gentle rotation for 10 minutes at room temperature. Magnetically separate and collect supernatant.
2. **Neutralization:** Immediately add 1/20–1/10 volume of Antibody Neutralization Buffer to the eluted antibody to restore neutral pH. This prevents antibody aggregation and maintains activity. The neutralized antibody can be used directly for SDS-PAGE and concentration measurement.

Step 6. Bead Storage

After use, wash beads twice with Bead Washing Buffer. Remove supernatant. Add 100 µL of Bead Storage Buffer. Store at 2–8°C.

Bead Regeneration Protocol

Repeated use leads to accumulation of precipitated proteins, hydrophobic proteins, and lipids on bead surfaces. Regenerate beads after 5 uses to maintain optimal performance.

1. Add 5 mL of Bead Regeneration Buffer per 1 mL of beads. Mix well and rotate for 10 minutes at room temperature. Magnetically separate and remove supernatant.
2. Wash twice with 5 mL of Bead Washing Buffer per 1 mL of beads, removing supernatant each time.
3. Resuspend beads in an appropriate volume of Bead Storage Buffer (1 mL per 1 mL original bead volume). Store at 2–8°C.

Important Precautions

1. Requires a magnetic separator (see Appendix 3 for compatible models).
2. Resuspend beads completely before each use by vortexing.
3. Store beads in supplied buffers—do not allow to dry.
4. DO NOT freeze or centrifuge beads—this causes irreversible aggregation.
5. Research Use Only (RUO)—not for diagnostic or therapeutic use.

Frequently Asked Questions (FAQ)

Q1: How can I improve antibody binding efficiency?

A1: Binding efficiency depends on antibody species/isotype and Protein A/G affinity (see Appendix 1). For low-affinity antibodies:

1. Increase incubation time (30–120 min)
2. Raise binding buffer pH (8–9)
3. Reduce ionic strength (25–100 mM NaCl)
4. Switch to Protein A/G beads for broader affinity.

Q2: How can I improve antibody elution efficiency?

A2: High-affinity interactions may reduce elution. Try:
Lower elution buffer pH (1.9–2.5)
Increase ionic strength (use 2–3 M $MgCl_2$)
Extend elution time.
Note: Low pH may cause aggregation—neutralize immediately with Tris or HEPES.

Q3: How do I prevent bead aggregation during storage/use?

A3: Store at 2–8°C. Add 0.1% (v/v) non-ionic detergent (NP-40, Tween-20, or Triton X-100) to Binding and Elution Buffers. If aggregation occurs after low-pH elution, neutralize with Binding Buffer and resuspend in Tris buffer (pH 7.5) containing 0.1% Tween-20, then sonicate for 2 minutes in a water bath. This does not affect binding capacity.

Q4: Beads stick to tube walls—how to prevent?

A4: Use low-protein-binding tubes. Add 0.01–0.1% (v/v) non-ionic detergent to buffers to reduce adhesion.

Q5: Beads clump together during use?

A5: Clumping occurs when beads remain on magnet too long. Use brief sonication (2 min) to disperse. WARNING: Do NOT sonicate after sample addition and before elution—this will release bound antibodies.

Appendices

Appendix 1: Affinity Comparison of Protein A and Protein A/G

Species	Antibody Class	Protein A	Protein G	Protein L	Recommend
Human	IgG1	+++	+++	+/- (κ)	A or G
	IgG2	+++	+++	+/- (κ)	A or G
	IgG3	+/-	+++	+/- (κ)	G
	IgG4	+++	+++	+/- (κ)	A or G
	IgM	+/-*	-	+++ (κ)	L (or A*)
	IgA, IgD, IgE	-	-	+++ (κ)	L
Mouse	IgG1	+	+++	+++ (κ)	G or L
	IgG2a	+++	+++	+++ (κ)	A or G or L
	IgG2b	+++	+	+++ (κ)	A or L
	IgG3	+++	+	+++ (κ)	A or L
	IgM	-	-	+++ (κ)	L
	IgA	-	-	+++ (κ)	L
Rat	IgG1	-	+	+ (κ)	G
	IgG2a	-	+++	+ (κ)	G
	IgG2b	-	+++	+ (κ)	G
	IgG2c	+++	+++	+ (κ)	A or G
Rabbit	IgG	+++	+++	-	A or G
Goat/Sheep	IgG	+/-	+++	-	G
bovine	IgG	+	+++	-	G
horse	IgG	+	+++	-	G
chiken	IgY	-	-	-	-
Recombinant antibody fragment	Fab/scFv/VHH	- (no Fc)	- (no Fc)	+/- (κ)	L or His tag

- +++: Strong binding
- +: Moderate or variable binding
- +/-: Weak binding or condition-dependent binding
- -: No binding or negligible binding
- (κ): Binds only antibodies with κ light chains (does not bind λ type)
- *: The J-chain region of some human IgM may exhibit weak binding to Protein A, but this is atypical.